Guide to Completing
The National Institute of Neurological Disorders and Stroke / National Institute of Deafness and Other Communication Disorders Animal Study Protocol Form (*NINDS/NIDCD ASP)

[*Appendix 1]

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Appendices

Introduction

The purpose of this guide is to help both new and seasoned investigators navigate the complex and sometimes onerous task of completing their animal study protocol forms. This is now done electronically at https://webapp.nhlbi.nih.gov/ninds-iaspapp/iasp.asp. The first part of this guide duplicates the electronic forms and provides help on completing each section. Don't be put of by the length of the guide; the first section covering protocol completion is the most important. The remainder of the guide contains appendices, sample entries, and other information that you will find useful.

Where possible, we have provided an explanation of terms and the numerous acronyms you will encounter when completing the forms. In the first part of the guide, we list the training necessary for Principal Investigators and other personnel on the proposal. If you have any questions or comments about the guide, or suggestions for clarification or improvement, please relay them to the Animal Health and Care Section Office at (301) 496-9354.

I. Section A – Administrative Data

Investigator requirements:

a. To become a **P**rincipal **I**nvestigator (PI) of an animal study protocol an NINDS scientist (not applicable to NIDCD researchers) must be one of the following: tenured, on tenure track, a Staff Scientist, or a Staff Clinician. This is NINDS policy and not **A**nimal **C**are and **U**se **C**ommittee (ACUC) policy.

b. When an investigator other than a PI plays a major role in the preparation and execution of a protocol, the ACUC recommends the designation of a **C**o-**P**rincipal **I**nvestigator (Co-PI). The PI, however, is ultimately responsible for any work done under his/her protocol.

c. All investigators listed in the study must fulfill training requirements (see Appendix 2).

- **General Requirements**

  - **Principal Investigators and Co-Principal Investigators must –**

    a) complete a lecture course on *Guidelines for Principal Investigators* conducted by the **O**ffice of **A**nimal **C**are and **U**se (OACU). Registration information, schedules, and all other information can be found on the Office of Animal Care and Use training page – http://oacu.od.nih.gov/index.htm.

    complete a web-based refresher course every three years. It is accessed using the same link as above.

    b) enroll in the **A**nimal **E**xposure **S**urveillance **P**rogram (AESP) with **O**ccupational **M**edical **S**ervices (OMS). It may be necessary to call OMS first for an appointment. See information on the OMS page - http://www.nih.gov/od/ors/ds/surveillance/animal.html.

  - **All other Co-Investigators (typically include post-docs, students, technicians, etc.) must –**

    a) complete the *Guidelines for Animal Users* conducted by the OACU. The refresher for this course must be taken every three years. Both the initial and refresher courses are web

based. See information on the OACU web page – http://oacu.od.nih.gov/index.htm.

b) enroll in the Animal Exposure Surveillance Program with Occupational Medical Services (OMS). To enroll in this program call OMS first to make an appointment. See information on OMS page - http://www.nih.gov/od/ors/ds/surveillance/animal.html

- **Additional Requirements Applicable to the Protocol Procedures –**

  - Training on using aseptic techniques is required for **all** investigators who are listed on a protocol that has a survival surgery procedure (see policy at http://ahcs.ninds.nih.gov/acuc/procedures.html). Survival surgery refers to surgery performed on an animal that subsequently recovers from anesthesia. This training is conducted by NINDS/NIDCD vets [Registration, schedule, and contact information can be found at the following link - http://ahcs.ninds.nih.gov/acuc/a_training.html.] The ACUC can grant exemption from taking the course if an investigator listed on a survival surgery protocol will not perform the surgery. Please note that the PI requests an exemption.

  - If the protocol uses non-human primates (NHP), investigators must complete *Working Safely with NHPs*. NINDS/NIDCD vets conduct this course. Please call the **A**nimal **H**ealth and **C**are **S**ection (AHCS) office at (301) 496-9354 to schedule an appointment.

  - The Division of Safety requires the completion of training in the safe handling of non-human primate tissue that may be contaminated with Blood Borne Pathogens (BBP). Please contact OMS at (301) 496-2346 for more information.

  - If the study requires euthanizing animals using cervical dislocation or guillotine without anesthesia, proficiency must be certified by NINDS/NIDCD veterinarians (see policy at http://ahcs.ninds.nih.gov/acuc/procedures.html). Please call the AHCS office at (301) 496-9354 to schedule an appointment.

  - Training in other protocol procedures must be indicated by the PI on the Investigator Training and Education Form (see Appendix 2.) NINDS/NIDCD veterinarians will train or supervise training when needed.

II. Section B – Animal Requirements

1. **Number of Years**
   The ACUC recommends requesting for three years instead of 1 or 2 unless the PI is certain that the study will be concluded within a year or two.

2. **Protocol Type**
   a. There are three animal study protocol types to choose from - *in vivo*, *in vitro*, and breeding. Any combination of these can be selected depending on the requirements of the study.

   b. *In vivo* protocol – a protocol in which procedures are done on live animals. All the *in vivo* procedures must be schematically represented in a flow chart (see Appendix 3 for examples of a flow chart).

   c. *In vitro* protocol – a protocol in which procedures or tissue removal is performed on an animal *after* euthanasia. Requested animal numbers have to be justified using an *in vitro* table or chart (see policy at http://ahcs.ninds.nih.gov/acuc/ExtentofDetailInVitroASPs.doc,. Examples of an *in vitro* table are provided in Appendix 4).

   d. Breeding protocol – a protocol in which animals are maintained and bred. It should be linked to a protocol defining the experimental use of the animals. A breeding chart must be used to justify requested animal numbers (see ACUC policy at http://ahcs.ninds.nih.gov/acuc/BreedingPolicy.pdf; see also Appendices 5, 6, and 7).

   e. If the protocol includes breeding, *in vivo*, and *in vitro* type procedures, all three - an *in vivo* flow chart, a breeding chart, and an *in vitro* table - should be attached. The charts/tables should be referenced in Section E.3 and Section F.

3. **Animal Numbers**

   a. Animal numbers must be entered for each year as required by the study. However, animal usage in a given year is not restricted to the number specified for that year in the protocol. Provided the total number of animals is not exceeded, investigators may use animals throughout the duration of the protocol as needed. An amendment is required if the need exceeds the total number approved for the study.

   b. For rodents, only weaned animals (older than 21 days) are counted.

   c. Pre-existing animals must be counted if the protocol is a renewal. Pre-existing animals are animals that will be transferred from the expiring

protocol to the renewal. Animals that are ordered or those that are physically housed under the expiring protocol are considered as pre-existing.

d. The animal numbers requested in this section have to match to animal numbers in other sections as follows:
   - They must match with the number in Section E.3 where justification for animal number is given.
   - They must match with the numbers in Section H assigned to United States Department of Agriculture (USDA) categories.
   - Depending on the type of protocol – the animal numbers in Section B must be consistent with the animal numbers in:
     - the flow chart for *in vivo* type,
     - the *in vitro* table for *in vitro* type, and
     - the breeding chart for breeding type.

4. **Species Data**

a. All species should be listed separately. It is advisable to list strains, especially when animals are immunocompromised or have other problems needing special attention/support of the AHCS.

b. The age range or weight of the animals should be specified, e.g. lactating mother with litter, juveniles, adults, pregnant mother, etc.

c. The source should be specified – vendor, in house, or specific information on importation from collaborators. If animals need to be imported or exported into or from NIH, specific procedures must be followed. Information on import and export procedures can be obtained at http://ahcs.ninds.nih.gov (see also Appendix 8).

d. The holding and procedure locations should be specified. This information (number of cages by species and where the animals can be housed - Bldg and room number) can be obtained from the AHCS office at (301) 496-9354. Procedure room locations (if different from the PI laboratory) can also be obtained from the AHCS office.

Cage allocation is not under the purview of the ACUC. You can find information on cage allocation from your Branch/Section chief or the Scientific Director.

e. PIs need to be aware of animal ordering procedures at NIH. The instructions can be found at http://ahcs.ninds.nih.gov (see also Appendix 9).

III. Section C - Transportation

NIH transportation guidelines must be followed – see http://oacu.od.nih.gov/ARAC/transport.pdf (see also Appendix 10). Animal holding facilities may have additional transportation requirements according to the building where the animal holding facility is located (Appendices 11 - 15). The transportation requirements of Bldg 36 and the Porter Neuroscience Research Center (PNRC) are a part of the facility orientation package. Please contact the Facility Manager at (301) 496-7108 for information.

Sample description:
"Transportation of animals will follow NIH transportation guidelines. All transportation of mice between the Building 36 SAF and Building 10NMR Center will be in NIH-approved disposable cardboard transport boxes with filter paper covering all openings/air vents. Rats will be transported from Building 10/5C127 using the NIH approved disposable cardboard transport boxes. The Clinical Center Animal Transportation Policy will be followed, including use of only the designated "animal only" elevator (Elevator# 28) to transport the animals between floors."

IV. Section D – Study Objectives

1. Objectives must be presented in terminology understandable to a lay person. Non-affiliated and non-scientific members of the ACUC should be able to understand the objectives.

2. The benefit of the study to human/animal health and/or to basic scientific knowledge must be described.

3. The goals of the study should be clearly defined and enumerated (if there is more than one goal) and linked to the appropriate experimental procedures (see examples in Appendix 16).

V. Section E – Rationale for Animal Use

1. Rationale for animal use – why use animals and not non-animal models, such as cell lines, computer models, etc.?

2. Appropriateness of species – the justification for using the selected species and no other. If more than one species, justification for each species must be provided separately.

3. Justification for requested animal number – there should be a justification for the requested animal number and not simply a list of animal numbers. If multiple species are used, then justify the numbers for each species separately.

   a. Justification of animal numbers for a **Breeding protocol** – the justification should be summarized in Section E.3 with detail in the breeding chart (see examples in Appendix 7).

   b. Justification of animal numbers for an ***in vivo* protocol** – the justification should be fully described in this section. Justifications can be statistical (e.g. statistical power), based on published results or on prior experience.

   c. Justification of animal numbers for an ***in vitro* protocol** – justification should be based on requirements of each *in vitro* project planned under the protocol (see appendix 4).

VI. Section F – Description of Experimental Design and Animal Procedures

1. All procedures that will be performed on animals (living or dead) must be described in this section within the context of the experimental design. Procedures include:
   a. Live Animals
      - minor and major survival surgery
      - multiple survival surgery (see policy on http://ahcs.ninds.nih.gov/policies/Multiple Survival Surgery.doc; Appendix 17)
      - non-survival surgery
      - behavioral or other testing
      - blood sampling
      - administering chemicals, drugs, radioactive materials, biological agents, DNA
      - genotyping (see policy – http://ahcs.ninds.nih.gov/policies/Rodent Tail Snip RevisedNovember02.doc; Appendix 18)
      - identification methods (see Appendix 18)
      - imaging (MRI, CT, PET) - If animals will undergo MRI procedures, PI should complete an NINDS NMR form (See Appendix 19), and the protocol should be reviewed by the NMR Center or the Mouse Imaging Facility (MIF). Contact the NMR/MIF for information at (301) 594-3898.
      - irradiation
      - animal restraint methods
      - *in vitro* experiments (brief discussion)
      - etc.

b. Dead Animals
An animal study protocol is required to use tissue collected from still-born animals or animals that were euthanized under a different protocol.

2. Post-procedure care of animals, in consultation with NINDS/NIDCD vets (if needed), should be summarized in this section with specific instructions in an Intervention and Endpoints table (see below for information on endpoints). Information on signs and symptoms with intervention assessments and treatment measures or endpoints should be specified in this table (see the example in Appendix 20). This table is used as a reference by animal holding facility vets and other facility staff who provide animal care.

3. The description of survival surgical procedures should be brief in this section. A detailed description should be provided in Section G.

4. All non-survival surgeries should be fully described in this section, not in Section G. Non-survival surgery is defined as any surgery performed on an animal that is subsequently euthanized without having recovered from general anesthesia.

Examples include:

a. cardiac perfusion for fixation.
Sample description:
Animals will be deeply anesthetized with a 5% isoflurane that is maintained at 2% throughout the procedure. Depth of anesthesia will be verified by pinching all four paws with forceps to make sure that there is no withdrawal reflex. The thorax will then be opened and animals will be transcardially perfused with 4% paraformaldehyde using aortic and right atrial cannulae in a closed system. The aorta (via left ventricle) and the right atrium will be cannulated so that a gravity-fed infusion of saline or PBS followed by paraformaldehyde perfuse the brain. The chemical waste will be collected as liquid waste per NIH waste disposal guidelines without being exposed to the atmosphere. Perfusion will be done in a chemical fume hood or on a down draft table. The person performing the procedure will wear a lab coat and gloves.
b. opening the uterus to remove fetuses followed by euthanasia of the mother.
c. terminal electrophysiology.

5. Description of blood withdrawal procedure is required, and the rationale for blood sampling. The description should distinguish between survival and terminal.

a. **Survival** – State whether sampling is single/serial, frequency of blood draw, blood volume, site of withdrawal, etc. See guidelines at http://oacu.od.nih.gov/ARAC/survival.pdf (See also Appendices 21 and 22).

b. **Terminal** – cardiocentesis should be described in this section together with the anesthesia that will be used and how the depth of anesthesia will be ensured (e.g. absence of withdrawal and blink reflexes). This procedure is considered USDA Category D procedure because it causes more than minimal or transient pain and/or distress but can be relieved using anesthetics, analgesics, sedatives, or tranquilizers (See Section H for more details of the various USDA categories). Such procedures require a literature search (see Section H) to ensure the absence of alternative procedures.

6. **Radioisotopes** – a description of the dose and route of administration with how a radioactive material will be used, and who will administer it to animals must be provided.
   a. Investigator must complete the training required by Radiation Safety before using radioisotopes. For training information, please see the Radiation Safety web page at http://www.nih.gov/od/ors/ds/rsb/hp/index.html.
   b. Radiation Safety must review and approve the protocol (see also Section K). The name of the radiation safety personnel can be found at http://www.nih.gov/od/ors/ds/rsb/hp/index.html or by calling Radiation Safety Branch at (301) 496-5774.
   c. Radiation Safety **S**tandard **O**perating **P**rocedure (SOP) must be followed Contact Radiation Safety at (301) 496-5774 for a copy of the SOP.
   d. If animals will be returned to the holding facility after a radiation procedure, the holding facility must be notified in advance about the use of radioactive materials.
   e. If using irradiators, the facility housing the irradiator should review and indicate its ability to support the protocol by signature. The location (Bldg. and room number) of the irradiator should be specified in Section B.

7. **Restraints** – any form of restraint should be described and the investigators involved should have the appropriate training. If training is needed, contact your veterinarian through the Animal Health and Care Section (AHCS) office at (301) 496-9354. Prolonged physical restraint must be justified scientifically and is considered an exception to policy. The method of restraint must also be described in Section M.
   a. restraint cannot be used as a method of housing animals, or for convenience.
   b. restraint is only used when it is an experimental requirement.
   c. the duration of restraint should be the minimum time required to meet the objective of the experiment.

8. **Drugs and Chemicals** - Drug and/or chemical classes (e.g. dopamine antagonists, glutamate agonists) should be stated together with the dose, volume,

concentration, route of administration, vehicle, and side effects. The dose, volume, concentration, and route of administration should be appropriate for the selected species and drug/chemical. If you are unsure, contact your veterinarian through the Animal Health and Care Section (AHCS) office at (301) 496-9354. If more than one drug/chemical is used, they should be listed in a table with the appropriate details (concentration, route of administration, side effects etc. (See Appendix 23). This requirement is intended to help address potential animal welfare concerns.

9. **Experimental Endpoint Criteria** – all studies must specify the end point of an experiment. Typically this will be euthanasia at a predetermined time. It is important to distinguish *experimental* endpoints (time points at which *in vivo* data collection are completed) from those required for medical reasons such as unrelieved pain or distress. *Medical* endpoints should be detailed in the intervention chart (see Appendix 20). In some cases medical and experimental endpoints may overlap.

VII. Section G – Survival Surgery

Survival surgery is surgery performed on an animal that subsequently recovers from general anesthesia.

1. A description of all survival surgery procedures (minor, major) must be provided in G.1.
   a. A detailed description of the survival surgery procedure/s and the aseptic surgery techniques that must be followed in any survival surgery must be described. Contact your consulting veterinarian if you need any assistance (See Appendix 25).
   b. Non-survival procedures should be described in Section F **not** G.

2. Individuals performing the surgical procedure(s) must be identified in G.2. These individuals must be fully trained in the procedure/s as supported by the training and experience form. If any training is required contact your veterinarian through the AHCS office at (301) 496-9354. Individuals who will perform survival surgery must complete the NINDS Aseptic Surgical Techniques Training (see http://ahcs.ninds.nih.gov/acuc/a_training.html; Appendix 26).

3. Identify the building and room where surgery will be performed together with Animal Health and Care Program requirements. Such program requirements include:
   a. Survival surgery on rodents can only be conducted on three days, Monday to Wednesday, unless special arrangements are made with AHCS veterinarians. This is necessary so that the recovery of the animals can be adequately monitored before the weekend.
   b. For animals that have undergone surgery, their cages should be flagged with a watch card and a yellow surgery card completed for each cage

when returned to their rooms after surgery. This is to identify them for follow up and monitoring.

c. The Principal Investigator should contact the facility veterinarian to learn the requirements for animals housed in non-NINDS managed facilities. For example, animals housed in Building 49 are managed by the National Eye Institute.

4. Post-operative care of animals and personnel who will provide this care must be specified in G.4.

5. If multiple survival surgeries are performed, the scientific justification should be explained in G.5 and G.6 (See Appendix 18).

VIII. Section H – Pain/Distress Category

1. All animals requested under a protocol must be assigned to applicable USDA pain/distress category/categories. There are three categories: **C, D** and **E**. Animals in categories D and E (see below) require a literature search to establish that no valid alternatives exist for any of the experimental procedures that involve the potential for more than momentary/transient pain or distress to the animals. At least two databases (e.g. Agricola, Medline) should be searched. Enter combination of key words into the search for which alternatives are being sought [e.g. 1) alternatives, pain, distress, C-section, mouse; 2) alternative, pain, distress, laminectomy, mouse; 3) alternatives, pain, distress, perfusion, rat; 4) alternative, pain, distress, thoracotomy; 5) alternative, cardiocentesis, pain, distress, mouse; etc.]. Provide a brief description of the search results. If alternatives are available but they are not appropriate for your study, state why they are not appropriate. The NIH library provides training and assistance in literature search for alternatives. Please see http://nihlibrary.nih.gov/ for information.

   a. **Category C** animals are those undergoing procedures that involve no, minimal or transient pain and/or distress. A search for alternatives is not required.
   Examples – all animals used for *in vitro* experiments belong to Category C since no procedure is performed before the animal is euthanized. Subcutaneous (SC), intra-peritoneal (IP), intravenous (IV), or intramuscular (IM) injections of drugs are examples of procedures that produce minimal/transient pain/distress. Also included in this category is anesthesia administered for euthanasia, animal restraint or imaging.

   b. **Category D** animals are those that undergo procedures causing pain and/or distress, which is relieved using anesthetics, analgesics, sedatives, or tranquilizers administered during and/or following the procedures.
      - A search for alternatives to the procedure causing pain/distress is required.

- Examples – all surgery including survival and non-survival surgery: craniotomy, tail snip of mice = 21days, exsanguinations, cardiac perfusion, etc.

c. **Category E** animals are those that undergo procedures in which pain and/or distress is *unrelieved* or that die as a result of such procedures before the experimental end point is reached.
- Scientific justification is required. The justification must be presented in a Column E form (Appendix 27). Category E animals are reported to the USDA.
- The number of animals in Category E should be minimized.
- Requires a search for alternative procedures (see above).
- Examples of studies that may result in Column E animals include those inducing **E**xperimental **A**utoimmune **E**ncephalomyelitis (EAE), seizure, cancer, or those that use death as an end point.

2. The animal numbers listed in this section should match with those listed in Section B, Section E.3, and on any additional documents such as Flow Charts or Breeding Charts.

IX. Section I – Anesthesia, Analgesia, Tranquilization

1. All anesthetics, analgesics, sedatives, tranquilizers should be described in this section. The dose/volume**,** concentration, and route of administration must be included in the description (refer to Appendix 24; please also contact a veterinarian if you need assistance). The preferred anesthetic for mice and rats is isoflurane. If this cannot be used for scientific or experimental reasons, please consult NINDS/NIDCD veterinarians. Barbiturates are not recommended for anesthesia due to prolonged recovery time. Topical anesthetics are recommended when using ear bars, at incision sites, etc.

2. The description provided in this section should match that of other sections, such as Section F or Section G which may contain references to anesthesia and analgesia. Gaseous anesthetics, such as isoflurane, must be listed in Section K as Hazardous Chemicals/Drugs.

X. Section J – Method of Euthanasia

Euthanasia is the act of inducing *humane death* in an animal. It should result in *rapid unconsciousness* followed by cardiac or respiratory arrest and ultimate loss of brain function. The technique should *minimize any stress or anxiety* experienced by the animal prior to unconsciousness (AVMA Panel on Euthanasia, 2000).

1. General Rules
   a. *Only trained personnel* should euthanize animals, using appropriate techniques, equipment, and agents. Some methods, such as cervical

dislocation, warrant special training and verification of skill. This includes AHCS technical staff and individuals listed on your protocol.

b. Animals should be euthanized in a non-public room where animals are not housed.

2. When to Euthanize
   a. Define the endpoints (both medical and experimental) for the study. Any study that may result in pain or distress requires an Intervention Table (see example in Appendix 20).
   b. If there is the potential for morbidity or death as an endpoint, please refer to the ARAC guideline titled *Endpoints in Animal Study Proposals* (see http://oacu.od.nih.gov/ARAC/endpoint.htm; Appendix 28).
   c. In solid tumor studies, animals should be euthanized if the tumor interferes with normal behavior, ulcerates, develops necrotic areas, or if it is not palpable but clinical signs such as weight loss, lethargy, or loss of appetite appear.
   d. Under **A**nimal **W**elfare **A**ct (AWA) guidelines, animals that would experience unrelieved severe or chronic pain or distress must be euthanized at the end of (or during, if necessary) the procedure. Any deviation must be scientifically justified and approved by the ACUC. Animals that experience unrelieved pain or distress are considered Column E requiring a Column E Explanation Form (Appendix 27) and must be reported to the USDA.

3. How to Euthanize
   a. **P**ublic **H**ealth **S**ervice (PHS) Policy states that the method of euthanasia must be consistent with the *2000 Report of the AVMA Panel on Euthanasia*. Appendix 1 of this text lists methods appropriate for various species. Any deviation from the text must be scientifically justified and approved by the IACUC.
   b. The animal's death **must** be verified before disposing of the carcass. Using injectable anesthetics, inhalant anesthetics, or carbon dioxide *requires* the use of a secondary form of euthanasia. For example, after using CO_2 inhalant, cervical dislocation, decapitation, or creation of bilateral pneumothoraces will insure the humane death of the animal.

4. Chemical Agents Appropriate for Euthanasia
   a. Carbon dioxide (inhalant)
      1) See *Guidelines for Euthanasia of Rodents Using Carbon Dioxide* at http://oacu.od.nih.gov/ARAC/euthanasia.pdf (Appendix 29) and *Guidelines for the Euthanasia of Mouse and Rat Fetuses and Neonates* at http://oacu.od.nih.gov/ARAC/euthmous.pdf (Appendix 30).
      2) The only acceptable source of carbon dioxide is compressed gas cylinders, and the National Institutes of Health requires the CO_2 source to be stated in the protocol.

3) A second, physical method of euthanasia such as cervical dislocation, decapitation, or creation of a bilateral pneumothorax should be used in order to ensure humane euthanasia.

b. Isoflurane, Halothane (inhalant anesthetic)
1) Potentially dangerous to humans and should only be used in a collection/scavenging system. These inhalants should also be listed as Hazardous agents in Section K of the Animal Study Proposal.
2) Requires a second, physical method of euthanasia such as cervical dislocation, decapitation, or creation of a bilateral pneumothorax in order to ensure humane euthanasia.

c. Injectable Anesthetics (pentobarbital, ketamine/xylazine)
1) Pentobarbital used in large animals (carnivores, ungulates, nonhuman primates) must be given intravenously and body parameters monitored (heart beat and withdrawal reflexes or respiration) until death is confirmed. Pentobarbital may be the most practical method for euthanizing non-human primates.
2) Pentobarbital used in rodents is given via intraperitoneal injection and a second physical method of euthanasia must be performed to ensure humane euthanasia.
3) Other anesthetic agents (ketamine/xylazine, chloral hydrate) are not suitable as euthanasia agents unless they are used in conjunction with a terminal procedure such as perfusion or cardiocentesis or tissue collection. In these cases, the animal must be deeply anesthetized (no withdrawal reflex, blink response, etc.) before beginning the procedure
4) Avertin used alone or as an anesthetic agent for perfusion or cardiocentesis is not acceptable because it is a poor anesthetic with minimal analgesic properties. Avertin's use is controversial and generally used only as a method of restraint or as the anesthetic for embryo transfer -- a procedure that takes less than five minutes by skilled personnel.

5. Methods of Euthanasia Requiring Anesthesia or Narcotization
a. Cervical Dislocation
1) Appropriate for mice and rats older than P14 and less than 200 grams, while anesthetized.
2) See *NINDS/NIDCD ACUC Policy on Cervical Dislocation* at http://ahcs.ninds.nih.gov/acuc/PolicyOnCervicalDislocation.doc (Appendix 32).

b. Decapitation
   1) See *NINDS/NIDCD ACUC Policy for Euthanasia of Mouse and Rat Fetuses and Neonates by Decapitation* at http://ahcs.ninds.nih.gov/policies/Euthanasia.htm (Appendix 31)
   2) Neonates (under P14) and fetuses may be decapitated with a sharp, heavy pair of scissors.
   3) Juveniles and adults can be euthanized by guillotine
      (a) Note the location of the guillotine and log book (building and room).
      (b) Follow the AHCS Standard Operating Procedure (SOP) 301 Maintenance of Guillotines (Appendix 33), and state that you will follow the SOP in Section J.

c. Cardiac Perfusion and/or Exsanguinations
   1) Animals must be **deeply anesthetized**. Physical parameters must be monitored to insure deep anesthesia and the procedure must be completely described.
   2) These procedures are *not* sole methods of euthanasia. They are considered a non-survival surgical procedure and must be described in Section F.
   3) Animals undergoing these procedures are considered Category D. A literature search for alternative procedures is required (see Section H).
   4) Aldehydes/picric acid used for perfusion must be listed in Section K.

6. Euthanasia without Anesthesia or Narcotization

a. Cervical Dislocation
   1) Cervical dislocation without anesthesia is allowed for rat and mouse fetuses and neonates below age P14.
   2) Cervical dislocation of rats and mice older than P14 without anesthesia is not allowed, unless;
      (a) There is scientific justification for performing the procedure without anesthesia.
      (b) The person performing this procedure should be named in Section J, and should also demonstrate his/her proficiency to NINDS/NIDCD veterinarian. If they are not deemed proficient, they must receive training until they are able to perform the procedure in a manner that is consistently humane. The protocol will not receive its final approval until the designated investigator is proficient in the technique.
   3) See *NINDS/NIDCD ACUC Policy on Cervical Dislocation* at http://ahcs.ninds.nih.gov/acuc/PolicyOnCervicalDislocation.doc (Appendix 32).

b. Decapitation
   1) Decapitation without anesthesia is allowed for rat and mouse fetuses and neonates below age P14.
   2) Decapitation of rats and mice older than P14 without anesthesia is not allowed, unless:
      (a) There is scientific justification for performing the procedure without anesthesia; and
      (b) a statement acknowledging adherence to Maintenance of Guillotines (Appendix 33).

XI. Section K – Hazardous Agents

1. **Radioactive Materials (Radionuclides)**
   a. All radioactive materials must be listed in this section.
   b. Protocols with radioactive materials must be submitted by the PI to the appropriate Radiation Safety personnel (health physicist) for review and approval. Radiation Safety assigns health physicists to conduct review of protocols that will use radioactive materials. Health physicists are assigned by building and floors where radioactive materials are used. The name of the health physicist can be located at http://www.nih.gov/od/ors/ds/rsb/hp/index.html or by calling Radiation Safety Branch at (301) 496-5774.
   c. The protocol must be approved by radiation safety before its final approval by the ACUC.

2. **Biological Agents**
   a. Potentially hazardous biological materials that will be transferred to live animals should be listed in this section (e.g. bacteria, pertussis toxin, botulinum, tetrodotoxin, ricin, picrotoxin, human/non-human primate cells, etc).
   b. If human pathogens, toxins, or human or non –human primate blood, body fluid, or tissues are to be used in the proposed animal study, the PI must first complete and submit a Human Pathogen Registration Document (HPRD), along with a draft copy of the Animal Study Proposal(ASP), for approval by the NIH Institutional Biosafety Committee(IBC) . A copy of the HPRD form may be found http://www.nih.gov/od/ors/ds/forms/hprd.pdf. Please also contact the NINDS/NIDCD Safety Officer at (301) 496-2346 for assistance.

   c. After receiving the IBC approval of the HPRD, the biological agents and HPRD number(s) and Animal Biosafety Level assigned by the IBC must be listed in Section K. The Animal Biosafety Level is assigned by the IBC and may be found on the IBC- approved HPRD. All applicable safety practices and procedures, equipment, personal protective equipment, and disposal methods required for work with the biological agents must be described in the designated space in Section K. Please also contact the

NINDS/NIDCD Safety Officer at (301) 496-2346 for more information approved safety practices.

3. **Hazardous Chemicals/Drugs**

a. All potentially hazardous chemicals or drugs (e.g. volatile or gaseous anesthetics, carcinogens, mutagens, teratogens) that will be used in the proposed animal study must be listed in Section K.

b. All applicable safety practices and procedures, equipment, personal protective equipment, and disposal methods required for work with the potentially hazardous chemicals or drugs must be described in the designated space in Section K.
Sample Descriptions:

- "Isoflurane will be used under a chemical fume hood (or scavenger system, or a local exhaust ventilation system) approved by the Division of Safety."
- "Formaldehyde will be used under a chemical fume hood (or scavenger system, or a local exhaust ventilation system) approved by the Division of Safety. Formaldehyde will be collected and disposed of as chemical waste following NIH approved safety guidelines."
- "Carcasses and animal tissue will be disposed of in MPW boxes following NIH approved safety guidelines."

Refer to the **NIH Chemical Hygiene Plan**, http://www.nih.gov/od/ors/ds/pubs/chp/chemhygplan03.pdf, for information on hazardous chemicals. Please also contact the NINDS/NIDCD Safety Officer at (301) 496-2346 for more information on approved safety practices.

c. The safety requirements for handling hazardous chemicals/drugs are described in the manufacturers **M**aterial **S**afety **D**ata **S**heets (MSDS). MSDS are required for hazardous chemicals (except for commonly used chemicals such as formaldehyde, gaseous anesthetics, etc.) and should be submitted with the animal study protocol. A list of searchable MSDS databases can be found at http://www.nih.gov/od/ors/ds/msds.html.

4. **Recombinant DNA**

a. When material containing rDNA is to be used in a proposed animal study, the work must be approved by the NIH Institutional Biosafety Committee (IBC). The PI must submit the following to the IBC:

1) A completed **R**ecombinant **D**NA (RD) form. This form is found at: http://forms.nih.gov/adobe/misc/NH2690.PDF.
Information on completing this form may be found at: http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html and

http://www.nih.gov/od/ors/ds/pubs/guide.htm Please also contact the NINDS/NIDCD Safety Officer at (301) 496-2346 for assistance.

2) map (s) of the DNA constructs
3) a copy of the animal study proposal
4) a completed HPRD form may be necessary.

c. The IBC meets once a month on the first Wednesday of the month. All Recombinant DNA applications must be received by the Committee at least *one week* before the meeting.

d. After receiving the IBC approval, the rDNA number(s) and the Animal Biosafety Level assigned by the IBC must be stated in Section K. The Animal Biosafety Level assigned by the IBC may be found on the IBC-approved rDNA. All applicable safety practices and procedures, equipment, personal protective equipment, and disposal methods required for work with rDNA must be described in the designated space in Section K. Please also contact the NINDS/NIDCD Safety Officer at (301) 496-2346 for more information on approved safety practices.

XII. Section L – Biological Material

1. Any use of biological products other than human or nonhuman primate (NHP) should be identified in this section.

2. A biological material must be tested when the historical details of tissue, tumor cell lines, or culture conditions (origins of serum, fibroblast feeder cells, etc.), such as microbiological conditions of the animals or animals used to generate these biological products, is unknown. **Test results must be attached to the protocol.**

3. To prevent accidental exposure of animals (especially rodents) to an infectious agent that may have contaminated a biological product, these materials derived must be tested according to the NINDS Policy regarding MAP, RAP, and HAP testing of Tissue Cultures and Biologics (see policy - http://ahcs.ninds.nih.gov/policies/MAPfinal.htm; Appendix 34).

   Definition:
   a. MAP – **M**ouse **A**ntibody **P**roduction Test
   b. RAP – **R**at **A**ntibody **P**roduction Test
   c. HAP – **H**amster **A**ntibody **P**roduction Test
   d. IMPACT – **I**nfectious **M**icrobe **P**CR **A**mplification **T**est

   These products must be tested to protect both the animal colony and human health.

4. A web link to recommended testing service - http://www.radil.missouri.edu/info/index.asp, or Contact the AHCS office at (301) 496-9354.

5. The PI certifies that the material has been tested and is uncontaminated by *initialing* this section.

6. If a human or NHP tissue or cell line will be used, an approved Human Pathogen Registration Document (HRPD) must be attached to the protocol, and referenced in Section K not in Section L.

XIII. Section M – Special Concerns or Requirements

All exceptions to policy, and concerns and special requirements should be discussed in this section. This section is referenced by facility managers to learn of any special requirements involved in housing animals. Any concerns should be described in this section to ensure that the facility staff knows what to do when situations arise. Examples include:

- Special feed (breeder chow, dough diets, mash feed, etc.)
- Food on cage floor (surgery, illness, etc.)
- Metabolic caging
- Single housing of animals
- Delayed weaning
- If the mother is pregnant (due to monogamous pairs, for example), pups *must* be weaned before the next litter arrives.

XIV. Section N – PI Certification

1. The PI signs in this section certifying that he/she is responsible for overseeing the work conducted under the protocol. Principal investigator responsibilities include:

   a. In Column D and E protocols, ensuring that no valid alternatives exist to the painful or distressful procedures.

   b. Ensuring the research is not unnecessarily duplicative.

   c. Verifying that personnel listed in Section A:
      - Are enrolled in the NIH AESP.
      - Have attended the "Using Animals in Intramural Research: Guidelines for Animal Users" and triennial refresher course.
      - Received training in the biology, handling and care of the appropriate species.
      - Are familiar with Aseptic Surgical Methods (if performing surgery)
      - Are trained in research and testing methods that limit the use of animals or minimize distress, as well as the proper use of anesthetics, analgesics, and tranquilizers.

- Know how to report animal welfare concerns

d. Obtaining ACUC approval ***before***:
   - Performing procedures with significant deviations from those described in the protocol.
   - Allowing new personnel to conduct procedures. Inform the ACUC when removing personnel, as well.

XV. Section O – Concurrences

1. The name and signature of the Laboratory/Branch Chief or the Scientific Director is required in this section, indicating approval of the study by the Institute. Normally, the signature of the Laboratory or Branch Chief is sufficient. However, if the PI is the Chief of a laboratory, branch, independent Unit, or independent Section, it is the Scientific Director who signs in this space.

2. The names of the current Safety Representative (if applicable), Radiation Safety Officer (if applicable), the animal holding facility location/s, facility manager/s, facility veterinarian/s, and the Attending Veterinarian must be entered in this section. The ACUC Coordinator will obtain the signatures after the ACUC reviews and approves the protocol. Contact the ACUC Coordinator by calling (301) 496-9354 for current names of the above personnel.

XVI. Section P – Final Approval

1. Name of the current ACUC Chairperson should be entered in this section.
2. The Chair signs after the Committee approves the protocol.
3. Contact the ACUC Coordinator by calling (301) 496-9354 for the name of the current ACUC Chairperson.

The NINDS/NIDCD Animal Study Proposal Form (ASP)

NATIONAL INSTITUTES OF HEALTH ANIMAL STUDY PROPOSAL
NINDS/NIDCD
(Adopted 03/00)(See NIH Manual 3040-2)

PROPOSAL#	
ACUC Review	
APPROVAL DATE	
EXPIRATION DATE	

A. ADMINISTRATIVE DATA:

Institute, Center, or Division: _____

Principal Investigator: _____ *E-Mail:*

Building: Room: Telephone: Fax:

Unit, Section, Laboratory, or Branch:

Project Title:

_____ *of Proposal*

List the names of all individuals authorized to conduct procedures involving animals under this proposal and identify key personnel (i.e., co-investigator(s)):

Name	AUPI	AUPI Refresher	AESP
No Co-Investigators			

B. ANIMAL REQUIREMENTS:

ASP Requested for: [ ] One Year [ ] Two Years [X] Three Years

Protocol Type: [ ] In Vitro ASP [ ] In Vivo ASP [ ] Breeding ASP

Comments:

Species Data

C. TRANSPORTATION:

Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported within the Clinical Center, also include the route and elevator(s) to be utilized.

Protocol Number

D. STUDY OBJECTIVES:

Briefly explain in non-technical terms the aim of the study and how the study may benefit human or animal health or advance scientific understanding of biological process.

E. RATIONALE FOR ANIMAL USE:

1) *Explain your rationale for animal use.*

2) *Justify the appropriateness of the species selected.*

3) *Justify the number of animals to be used. (Use additional sheets if necessary.)*

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES:

Briefly explain the experimental design and specify all animal procedures. This description should allow the ACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. Specifically address the following: (Use additional sheets if necessary.)

- *Injections or Inoculations (substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules)*
- *Blood Withdrawals (volume, frequency, withdrawal sites, and methodology)*
- *Non-Survival Surgical Procedures (provide details of survival surgical procedures in Section G.)*
- *Radiation (dosage and schedule)*
- *Methods of Restraint (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)*
- *Animal Identification Methods (e.g., earpunches/notches, ear tags, tattoos, collar, cage card, etc.)*
- *Other Procedures (e.g., survival studies, tail amputations, etc.)*
- *Resultant Effects, if any, the animals are expected to experience (e.g., pain or discomfort, ascites production, etc.)*
- *Experimental Endpoint Criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified.*

G. SURVIVAL SURGERY - IF PROPOSED, COMPLETE THE FOLLOWING:

1. *Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized.(Use additional sheets if necessary.)*
2. *Who will perform surgery and what are their qualifications and/or experience?*
3. *Where will surgery be performed: Building/Room:*
4. *Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:*
5. *Has major survival surgery been performed on any animal prior to being placed on this study? If yes, please justify: ____*
6. *Will more than one major survival surgery be performed on an animal while on this study? If yes, please justify: ____*

H. PAIN OR DISTRESS CATEGORY :

The ACUC is responsible for applying U.S. Government Principle IV. Contained in Appendix 3: "Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals." Check the appropriate category(ies) and indicate the approximate number of animals in each. Sum(s) should equal total from Section B.

IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED TO EXPLAIN WHY THE USE OF ANESTHETICS, ANALGESICS, SEDATIVES OR TRANQUILIZERS DURING AND/OR FOLLOWING PAINFUL OR DISTRESSFUL PROCEDURES IS CONTRAINDICATED. PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO THE USDA. NOTE: THIS COLUMN E FORM, AND ANY ATTACHMENTS, e.g., THE ASP, ARE SUBJECT TO THE FREEDOM OF INFORMATION ACT.

Describe your consideration of alternatives to procedures Listed for Column D and E that may cause more than momentary or slight pain or distress to the animals, and your determination that alternatives were not available. [Note: Principal Investigators must certify in paragraph N.5. that no valid alternative was identified to any described procedures which may cause more than momentary pain or distress, whether it is relieved or not.] Delineate the methods and sources used in the search below. Database references must include databases (2 or more) searched, the date of the search, period covered, and keywords used:

No Databases Searched

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION:

For animals indicated in Section H, Column D, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route and schedule of administration.

Protocol Number

J. METHOD OF EUTHANASIA OR DISPOSITION AT END OF STUDY:

Indicate the proposed method, and if a chemical agent is used, specify the dosage and route of administration. If the method(s) of euthanasia include those not recommended by the AVMA Panel Report on Euthanasia, provide justification why such methods must be used. Indicate the method of carcass disposal if not as MPW.

K. HAZARDOUS AGENTS:

Use of hazardous agents requires the approval of an IC safety specialist. Registration Documents are required to be attached for the use of recombinant DNA or potential human pathogens may be attached at the discretion of the ACUC.

		List agents and registration document number (if applicable)
[___]	*1. Radionuclides*	
[___]	*2. Biological Agents*	
[___]	*3. Hazardous Chemicals/Drugs*	
[___]	*4. Recombinant DNA*	

Study conducted at Animal Biosafety Level

Describe the practices and procedures required for the safe handling and disposal of contaminated animals and material associated with this study. Use of volatile anesthetics requires a description of scavenging methods used. Also describe methods for removal of radioactive waste and, if applicable, the monitoring of radioactivity.

Additional safety considerations:

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS (e.g., cell lines, antiserum, etc.):

1. *Specify:*
2. *Source: Material Sterile or Attenuated? ____*
3. *If derived from rodents, has the material been MAP/RAP/HAP/PCR tested? _____ (Attach copy of results)*
4. *I certify that the MAP/RAP/HAP/PCR tested materials to be used have not been passed through rodent species outside of the animal facility in question and/or the material is derived from the original MAP tested sample. To the best of my knowledge the material remains uncontaminated with rodent pathogens.*

_____ *Initials of Principal Investigator.*

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY:

Protocol Number

List any special housing, equipment, animal care (i.e., special caging, water, feed, or waste disposal, etc.). Include justification for exemption from participation in the environmental enrichment plan for nonhuman primates or exercise for dogs.

Protocol Number

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS:

1. ***I certify that*** *I have attended an approved NIH investigator training course.*

   Year of Course Attendance Location

   Year(s) of Refresher Training:

2. ***I certify that*** *I have determined that the research proposed herein is not unnecessarily duplicative or previously reported research.*

3. ***I certify that*** *all individuals working on this proposal who have significant animal contact are participating in the NIH Animal Exposure Surveillance Program.*

4. ***I certify that*** *the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal have attended the course "Using Animals in Intramural Research: Guidelines for Animal Users" and will complete refresher training as required, and received training in the biology, handling, and care of the species; aseptic surgical methods and technologies (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); procedures for reporting animal welfare concerns.*

5. ***FOR ALL COLUMN D AND COLUMN E PROPOSALS (see section H): I certify that*** *I have reviewed the pertinent scientific literature and the sources and/or databases (2 or more) as noted in paragraph H, and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not*

6. *I will obtain approval from the ACUC before initiating any significant changes in this study.*

Principal Investigator Signature: _____ *Date:* _____

Protocol Number

O. CONCURRENCES: PROPOSAL NUMBER - _____

Laboratory/Branch Chief *certification of review and approval on the basis of scientific merit. Scientific Director's signature required for proposals submitted by a laboratory or branch chief.*

Signature	*Date*

Safety Representative *certification of review and concurrence. (Required of all studies utilizing hazardous agents.)*

Signature	*Date*

Radiation Safety Officer *certification of review and concurrence. (Required of all studies utilizing radioactive materials.)*

Signature	*Date*

Facility Manager *certification of resource capability in the indicated facility to support the proposed study.*

Facility	*Signature*	*Date*

Facility Veterinarian *certification of review.*

Protocol Number

Facility	*Signature*	*Date*

Attending Veterinarian *certification of review.*

	Signature	*Date*

Protocol Number

P. FINAL APPROVAL

Certification of review and approval by the NINDS/NIDCD Animal Care and Use Committee Chairperson.

CHAIRPERSON

Signature *Date*

NINDS/NIDCD Investigator Training and Experience for Animal Studies

NINDS/NIDCD Investigator Training and Experience for Animal Studies

Name of Investigator:		*ASP #*
Email Address:		
Bldg, Room, and Mail Stop numbers:		
Telephone number:		
Fax number:		
1	*What is your academic background?*	
2	*What is your research/employment position at NIH (IRTA, Research Fellow, Special Volunteer, Technician, etc.)?*	
3	*What species of animals have you worked with?*	
4	*Are you trained to perform surgical procedures on animals using aseptic techniques?* YES ☐ NO ☐	
5	*What other techniques have you been trained to perform on animals (decapitation, tail vein bleed, tail snip, plug check, etc.)?*	
6	*What surgical procedures have you performed?* *Survival* ☐ *Non-survival* ☐ *None* ☐ *In what animal species?*	
7	*Please specifically state the protocol procedure/s you will perform on animals:*	
8	*Are you trained to perform the above procedures?* YES ☐ NO ☐	
9	*Who will supervise and/or train you in the protocol procedures?*	
10	*Training/enrollment:* ***please checkmark the boxes where applicable. The ACUC Coordinator will fill in the dates.***	
	Guidelines for Animal Users ☐ Date:	**Guidelines for Principal Investigators* ☐ Date:
	Animal Use Refresher Course ☐ Date:	*PI Refresher Course* ☐ Date:
	Enrollment in the Animal Exposure Surveillance Program (AESP) ☐ Date:	
	The NINDS/NIDCD Aseptic Techniques course if survival surgery is performed under this protocol ☐ Date:	
	Working Safely with NHP course if this is a Nonhuman Primate(NHP) protocol: ☐ Date:	

Note: *If this person is being added to an already approved protocol, will the addition of this person change the Disposition Instructions Form?* YES ☐ NO ☐ *(If **yes**, please submit new Disposition Instructions Form)*

Investigator signature below indicates that s/he has read and understands the protocol

Name of Principal Investigator:	Signature:	Date:
Signature of Investigator :	Date:	

CONCURRENCES

Attending Veterinarian: Judith Davis, DVM, MS	Signature:	Date:
Chairperson: Judith Walters, Ph.D.	Signature:	Date:

*Principal Investigators and Co-Principal Investigators must take the Guidelines for Principal Investigators.

Example Flow Charts For *In Vivo* Studies

EXAMPLES OF *In Vivo* Flow Charts

Example 1

Total number of animals = 60

I. Uptake of ^{3}H-steroid in brain tumors vs normal brain (n=10 animals)

Implant tumor cells, Day 0

Cell line #1 (n=5)
Day 16

Cell line #2 (n=5)
Day 9

At indicated day, animals are anesthetized, vessels cannulated,
and given i.v. bolus of ^{3}H-steroid (250µCi)

collect blood samples: 10,20,30,45 min

Euthanize animals, harvest brain and lung for autoradiography and immunohistochemistry

II. Steroid as regulator of BBB function

A. Determine optimal time of steroid pretreatment (n=16 animals)

Implant tumor cells, Day 0

Cell line #1 (n=8)
Day 15

Cell line #2 (n=8)
Day 8

i.p. injection of steroid (3 mg/kg) (n=5) or vehicle (n=3)

16 hours

The next day, animals are anesthetized, vessels cannulated,
and given i.v. bolus of ^{14}C-permeability tracer (100µCi/animal)

collect blood samples: 1,3,5,10,15 min

Euthanize animals, harvest brain for autoradiography and immunohistochemistry

B. Does steroid pre-treatment alter BBB transporter activity? (n=20 animals)

Implant tumor cells, Day 0

Cell line #1 (n=10)
Day 14 or15

Cell line #2 (n=10)
Day 7 or 8

i.p. injection of steroid (n=5/tumor type) or vehicle (n=5/tumor type)

16 (or 48) hours as determined in part II A

On day 16 (Cell line #1) or 9 (Cell line #2), animals are anesthetized, vessels cannulated, and given i.v. bolus of ^{3}H-transporter substrate (100uCi/animal)

collect blood samples: 10,20,30,45 min

Euthanize animals , harvest brain for autoradiography and immunohistochemistry

III. Does inhibition of BBB transporter alter steroid uptake? (n=14 animals)

Implant tumor cells, Day 0

Cell line #1 (n=7) Day 16

Cell line #2 (n=7) Day 9

i.p. injection of BBB transport inhibitor drug (30 mg/kg) (n=5) or vehicle (n=2)

Wait 60 min

Animals are anesthetized, vessels cannulated, and given i.v. bolus of ^{3}H-steroid as in "Part I" (250µCi/animal)

collect blood samples: 10,20,30,45 min

Euthanize animals, harvest brain and lung for autoradiography and immunohistochemistry

EXAMPLE 2

Survival Surgery
(Hepatectomy and/or Hepatocyte Transplantation)
(Mice used: Alternate between Mick 1, Mick 3, 2110, 2111, or B6D2)
Mice are on regular lab chow
or
3-4 days prior to surgery, placed on Liquid Diet and/or Jell-O + 0.5-5% Creatine

N = 3 mice/week
Anesthetize mice with Isoflurane

OR

OR

65% Hepatectomy
(continue creatine diet if needed)

65% Hepatectomy
with coinciding
Hepatocyte Transplantation into spleen (200 ul)
(continue creatine diet if needed)

Hepatocyte Transplantation into spleen (200ul)
(continue creatine diet if needed)

. or 2 mice: 12 hours post surgery: Perform MRI on
nesthetized mouse and/or remove tissues for biochemical
nalysis. Animal/s is/are sacrificed.

. or 2 mice: 24 hours post surgery: Perform MRI on
nesthetized mouse and/or remove tissues for biochemical
nalysis. Animal/s is/are sacrificed.

. or 2 mice: 36 hours post surgery: Perform MRI on
nesthetized mouse and/or remove tissues for biochemical
nalysis. Animal/s is/are sacrificed.

. or 2 mice: 48 hours post surgery: Perform MRI on
nesthetized mouse and/or remove tissues for biochemical
nalysis. Animal/s is/are sacrificed.

1-6 mice: 1-4 days post transplantation. Anesthetize mice with Isoflurane. Perform MRI on anesthetized mouse with non-invasive temperature monitoring and EKG, to check liver regeneration. Allow to recover for future imaging. Tissues may be removed on mice that do not recover from anesthesia and used for biochemical analysis.

1-6 mice: 5-8 days post transplantation. Anesthetize mice with Isoflurane. Perform MRI on anesthetized mouse with non-invasive temperature monitoring and EKG, to check liver regeneration. Allow to recover for future imaging. Tissues may be removed on mice that do not recover from anesthesia and used for biochemical analysis.

1-6 mice: 9-11 days post transplantation. Anesthetize mice with Isoflurane. Perform MRI on anesthetized mouse with non-invasive temperature monitoring and EKG, to check liver regeneration. Allow to recover for future imaging. Tissues may be removed on mice that do not recover from anesthesia, and used for biochemical analysis.

1-6 mice: 12-14 days post transplantation. Anesthetize mice with Isoflurane. Perform MRI on anesthetized mouse with non-invasive temperature monitoring and EKG, to check liver regeneration. Animals sacrificed no later than 14 days post surgery using KCl, IV, to effect. Tissues may be removed for biochemical analysis.

Examples of *In Vitro* Table

EXAMPLES OF *IN VITRO* TABLE

EXAMPLE 1

Table of *In Vitro* Projects

Project Title	Animals/unit time	No. of Investigators	Description
Dynamics of glutamate uptake into hippocampal astrocytes	RATS: year 1: 0.3 litter/week year 2: 0.4 litter/week year 3: 0.2 litter/week MICE: year 1/2: 0 litter/week year 3: 0.4 litter/week	1	Electrophysiological recordings from hippocampal astrocytes are used to determine the time course of glutamate uptake following synaptic release. Will use rats and eventually transgenic mice expressing fluorescent transporters.
Modulation of glial glutamate transporters in the hippocampus	RATS: year 1/2/3: 0.4 litter/week MICE: None.	1	Electrophysiological recordings from rat hippocampal astrocytes and neurons are used to study the modulation of glutamate transporters by synaptically released glutamate.
Roles for glutamate transporters in inhibitory synaptic terminal in the hippocampus	RATS: year 1: 0.3 litter/week year 2/3: 0.2 litter/week MICE: year 1: 0 litter/week year 2/3: 0.5 litter/week	1	Electrophysiological recordings from hippocampal pyramidal cells are used to study roles of glutamate transporters in supplying inhibitory synaptic terminals with glutamate, a substrate for GABA synthesis. Will use rats and transgenic transporter-knockout mice.
Synaptic excitation of ganglion cells in the mammalian retina	RATS: year 1/2: 0.4 litter/week year 3: 0.2 litter/week MICE: year 1/2: 0 litter/week year 3: 0.4 litter/week	1	Electrophysiological recordings from ganglion cell in retinal slices are used to study the activation of ganglion cell glutamate receptors in response to electrical and light stimulation. Experiments in rats and transgenic mice (transporter KO).
Dynamics of synaptic transmission at a mammalian ribbon synapse	RATS: year 1/2: 0.4 litter/week year 3: 0 litter/week MICE: year 1/2: 0.2 litter/week year 3: 0.4 litter/week	1	Dual electrophysiological recordings between synaptically coupled bipolar cells and amacrine cells are used to study the dynamics of synaptic release and depression. Will use rats and transgenic mice (GFP constructs).
Optical studies of synaptic transmission in the CNS	RATS: year 1/2/3: 0.2 litter/week MICE: year 1/2/3: 0.3 litter/ week	1	Electrophysiological recordings are combined with multiphoton laser scanning microscopy to investigate the physiology of single identified synapses. Rats and transgenic mice (various constructs) will be used.

For both species, litter/week calculation is based on estimate of 8 pups per litter.

EXAMPLE 2

Table of *In Vitro* Projects

Project Title	**Animals/unit time**	**# Investigators**	**Description**
1. Role of creatine kinase in cardiac metabolism	8mice/week	1	Physiologic function and metabolism is monitored during normal, ischemic, and oxidative stress using the perfused heart.
2. Identification of changes in the Mitochondrial proteome	8mice/week	1	2D gel analysis is used to identify and monitor changes in mitochondrial proteins using isolated mitochondria of the heart, liver, or brain.
3. Role of creatine kinase in hepatocyte metabolism	3mice/week	2	Effects of creatine kinase and physiological function and cell growth are studied in isolated and perfused livers.

NINDS/NIDCD Animal Care and Use Committee (ACUC) Policy for the Extent of Detail Required in Breeding Animal Study Proposals (ASPs)

NINDS/NIDCD Animal Care and Use Committee (ACUC) Policy for the Extent of Detail Required in Breeding Animal Study Proposals (ASPs)

A major consideration in reviewing ASPs is promotion of animal welfare through use of numbers appropriate to experimental goals; therefore the Committee requires a breakdown of animal use within breeding protocols. Five categories of use must be separately enumerated: 1) numbers used for breeding (including founders, background strain, and retained progeny), 2) total numbers of expected progeny, 3) the numbers of progeny intended for experimental use or export, 4) the numbers of progeny needed for continuation of the experimental line, and 5) the numbers that will be euthanized due to undesirable genotype. Animals with undesirable genotype are not counted into animal number totals if these animals are euthanized before weaning, but for completeness should be listed in the numerical breakdown. These categories should be enumerated both on a single generation basis and on an annualized basis.

In addition, principal investigators must present the Committee with objectives for the breeding (i.e. homozygous pups for experiments, or back crossing to establish a genetic mutant on a homogeneous background). Enough detail of the breeding schemes must be included to allow the Committee to assess the appropriateness of requested animal numbers. Examples are provided based on commonly employed breeding schemes. These examples include suggested breeding assumptions to use for new strains where breeding parameters may be unknown, but an animal number must be provided for purposes of review. An outline, a table, or a flow chart must be provided for each strain listed in a breeding ASP. This information should be included under Section F as the experimental design and referenced in Section E.3.

Examples of Breeding Schemes

Glossary

Mutant = knock-out or Transgenic mouse.
Wild type (+/+) = pups from mutant crosses that do not carry the gene of interest at either allele, or background strain that does not carry a mutation.
Heterozygous (het, Aa, or +/-) = the mutant gene of interest is carried as one dominant allele and one recessive allele.
Homozygous (hom, AA, or aa) = the mutant gene of interest is carried as either dominant or recessive at both alleles.
Background strain = the inbred strain or strains on which the mutant gene was established, e.g. C57BL/6 or 129.
Congenic strains = two strains that are genetically identical except for a short chromosomal segment, achieved by backcrossing to an inbred strain (usually 10 backcrosses).

A. Maintaining an Established Mouse Strain/Line

When predicting the number of mice required in maintaining an established strain, actual breeding data is used or certain assumptions are made as follows:

- Age of new breeders – both male and female usually mature at 2 months of age.
- Number of pups per litter – 5 pups/litter if average litter size is unknown.
- Number of litters per female – female will produce 4 litters with no postpartum breeding and is typically retired after 6 months.

Example: 2 breeder pairs X 4 litters/each X 5 pups/litter = 40 pups

B. Mice needed for general experiments

After predicting the number of pups that a breeding pair will produce, it will be necessary to determine how many of the pups will carry the gene of interest and then work backwards to determine the number of breeding pairs needed to support the research. The number of mice needed for an experiment may depend on the phenotype, background strain variability, etc., that is known or thought to occur in the mice. Therefore, it may be useful to include a percentage of all pups, rather than only homozygous mutants (-/-) in the experimental design.

- Recessive or Dominant Mutant (a/a or A/A) maintained via het X het breeding
  Y = number of mutant mice / experiment
  25% of pups will carry gene of interest as (-/-)
  Y/0.25 = total pups needed

Example: Let Y = 100
100 / 0.25 = 400 pups
Assume 5 pups/litter X 8 litters/yr = 40 pups/pair/yr
Therefore: 400 pups/40 = 10 breeder pairs/yr (or 20 breeders total)
Total mice: 400 pups + 20 breeders = 420 mice

- Recessive or Dominant Mutant (a/a or A/A) maintained via het X hom breeding

  Y = number of mutant mice / experiment
  50% of pups will carry gene of interest as (A/- or a/-)
  Y/0.50 = total pups needed

  Example: Let Y=100
  100 / 0.5 = 200 pups
  Assume 5 pups/litter X 8 litters/yr = 40 pups/pair/yr
  Therefore: 200 pups/40 = 5 breeder pairs/yr (or 10 breeders total)
  Total mice: 200 pups + 10 breeders = 210 mice

C. Mice needed to generate a congenic line (homogeneous background)

When establishing a new mutant model, it is usually desirable to maintain it on a homogeneous background strain. Many mutants are created on a mixed strain background (usually B6 and 129) that ultimately can interfere with interpretation of experimental results. If the mutant is on a mixed background, a series of backcrosses will both stabilize the allelic position of the mutation (if transgenic) and create a homogeneous background strain. Ideally 10 backcrosses should be performed, which will achieve 99.8% homogeneity. At a minimum, 4 backcrosses should be performed to achieve 94% homogeneity.

Assumption: will maintain two backcross breeding pairs per generation, will produce 5 pups per breeder pair or 10 pups total per generation, and will screen prior to weaning (*if not, then must count +/+ pups in total*).

Breeding scheme:

Donor het x background strain (+/+)	= N1
N1 het X (+/+)	= N2
N2 het X (+/+)	= N3
And so on until N10	

Example:
50% of pups will carry gene of interest as het (+/-)
10 pups X 0.5 = 5 mice / generation
5 mice/generation x 10 generations = **50** donor mice

Need two background strain (+/+) mice / generation
2 x 10 generations = **20** background mice
(Also **2** original donor mice)

Total = 50 + 20 + 2 = 72 mice
Total if screen after weaning: 50 + 20 + 2 + 50(+/+ pups) = 122 mice

D. Total mice accountability

In addition to the total number of mice needed for an experiment, there must be accountability for the following mice:

- Breeding mice – when mice are bred to maintain a line or produce animals for experiments, some will need to be held back to replenish the breeding pool.
- Cull mice – if pups are genetically screened and culled prior to weaning, they DO NOT have to be counted towards the total number. If genetic screening is performed at or after weaning, ALL MUST be counted toward the total number listed on the protocol.
- Remember, female breeders are usually retired after 6 months.

Example: 2 heterozygous breeder pairs X 4 litters X 5 pups = 40 pups + 4 breeders = 44 mice;

- 10 (-/-) are desired mutants;
- 8 (+/+) used for negative control;
- 2 (+/-) females used to replenish breeding stock at 6 months; and
- 2 (+/+) and 18 (+/-) are euthanized.

Breeding Protocol Talking Points

Breeding Protocol Talking Points

Animals ≥ P21 days are counted; < P21 are not counted
Must describe how you plan to breed the animals:

Permanent pairs – litters every 21 days (approximately)

Issues: Must wean existing litter before next litter drops*
Exhausts the female
Consider using breeding chow

Harem Breeding – 1 male with 2 or 3 females (no more)

Issues: Plug checks necessary if want to know females are pregnant
Must separate pregnant females by E16
Some strains, males unreliable performers under this strenuous breeding paradigm (e.g. can't handle multiple females within narrow time frame)

Temporarily Pair – put female with male

Issues: Must do plug check

Breeder Replacement – females every 6 mo, males every 12 mo
Genotyping

Tail Snip ≤ 0.2 cm first snip (NIH/ARAC Guidelines)

Issues: Use of anesthesia (depends on age of tail snip)
Method of identification of pups (age of tail snip)

Tips:

If ≤P7 days: can use tattoo or digit (toe) snip to ID pups
Do not need to use anesthesia (but encouraged)
Scientific justification for digit snip required
Advantage: cull WT pups early – better for wanted pups

If >P7 but < P14 days: topical anesthetic minimum (ethyl chloride popular)
AHCS recommends isoflurane (faster)
ID method depends on age of tail snip (maturity of cartilage)
Can not use digit snip as method of ID

If ≥ P21 days: Must use general anesthesia (recommend isoflurane)
Category D procedure
Alternative Search (Section H) required

Recommend adding a phrase in Section M that the first "snip" will be < 0.2 cm but in case something happens to the DNA or assay, may need to re-snip; in that event, a **total** of tail removed (e.g. including the first snip) will be less than 0.5 cm total.

Weaning

Must wean at P21 and separate by gender (4 mice/cage)

If pups are small (< 16 gm) can request (**Section M**) "exception to policy" to delay weaning until P28 days of age

This exception to policy applies only to small pups, not necessarily entire litters

If the mother is pregnant, the pups **must be weaned** before the next litter drops

Breeding Chart Required

Each line described (heterozygous, homozygous breeders or backcross)
Type of Mating (permanent pairs, harem, temporarily pair)
Average litter size exp ect from each line; age expect to replace breeders
Fate of pups – must tie-in with aims/objectives (**Section D**)
Total number of breeders/line + number of pups weaned (counted)
Numbers in chart must mathc Sections B, E.3, H, Flow Chart, *in vitro* chart

Identification

Ear cartilage does not mature sufficiently until P18 – P21 to support ear tags
Use of ear tags or ear punch before P18 often leads to later problems
Common identification systems used:

Ear tag
Ear Punch/Notch
Tattoo (recommended)
Transponder (excellent but expensive)
Digit (toe) snip – only if pups are altricial (no hair) – less than 7 days old

References:

1) NIH Guidelines for the Genotyping of Rodents
2) NINDS/NIDCD Animal Care and Use Committee (ACUC) Policy for Rodent Tail Snip and Altricial Pup Identification"
3) NIH ARAC "Recommendation for Toe Clipping in Animals"

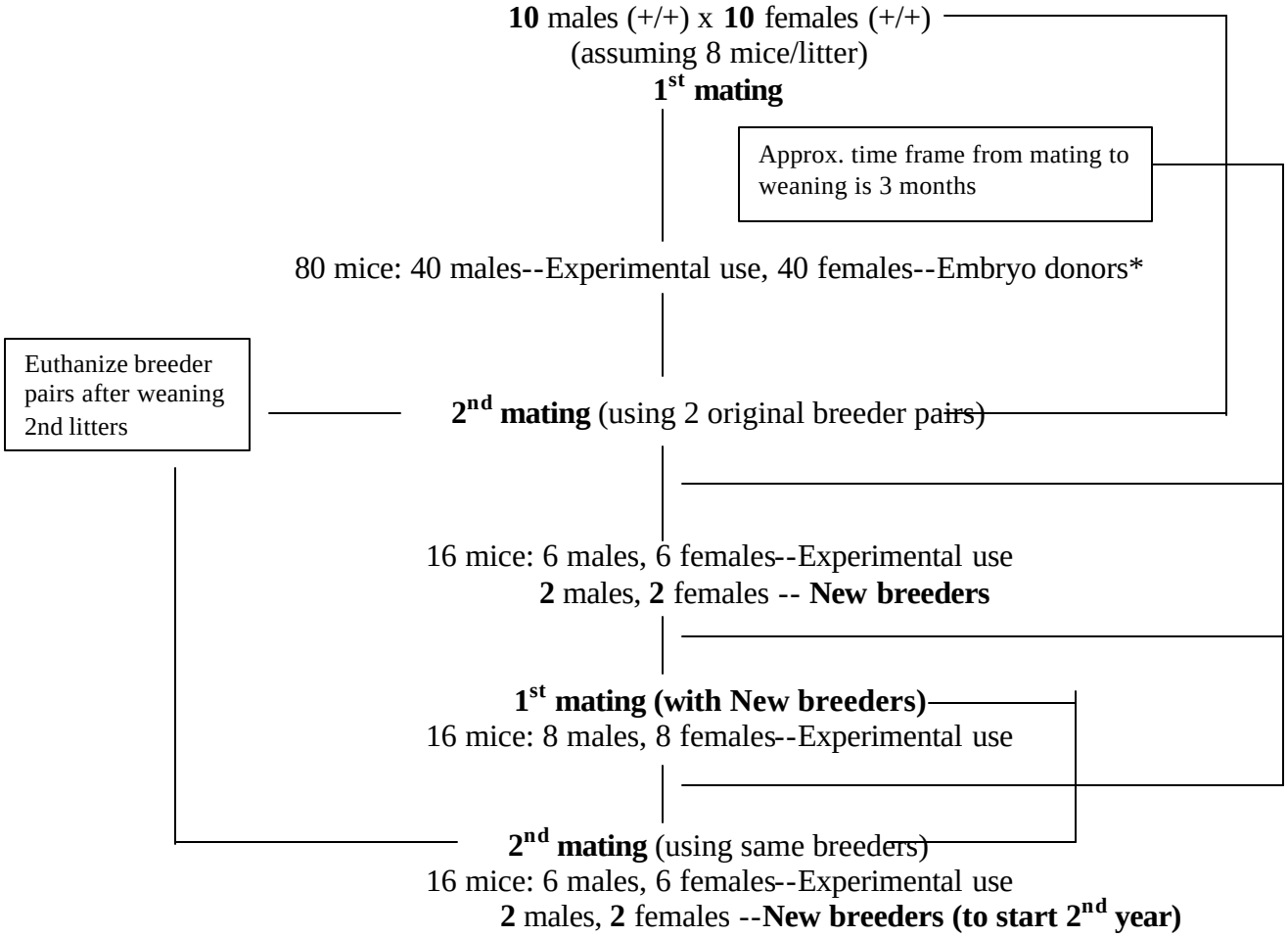
Examples of a Breeding Chart

EXAMPLES OF BREEDING CHARTS

EXAMPLE 1

Breeding for Homozygote Lines (with Good reproducing Ability)
Homo 2110, Homo MiCK 3, Homo PATS 6, B6D2

(+/+) Homozygote; (+/-) Heterozygote; (-/-) Wild Type



80 mice/for Exp/year #1/line x 4 lines = 320 mice/year #1
28 mice/as Breeders/year #1/line x 4 lines = 112 mice/year #1*

Assuming that the lines have been successfully rederived, Year #2 & #3 will follow the breeding scheme as shown on the second half of flow chart (time frame is 6 months, so double the numbers for 1 year)

56 mice/for Exp/year x 4 lines = 224 mice/year
8 mice/as Breeders/year x 4 lines = 32 mice/year **

***Embryo donors are mated to original male breeders. Embryo transfers are done according to ASP 988-00.**

****Numbers used for this protocol**

EXAMPLE 2

Line	Purpose	Numbers
Total	**Breeding**	**361**
Balb/cJ	Used for backcrosses	Currently 0, anticipate fewer than **4** per year
129/SvJ	Used for backcrosses	Currently 0, anticipate fewer than **4** per year
C57BL/6	Used in backcrosses outlined below bought from Jackson labs	**25**
B6C3Fe-a/a-Reln	Maintenance of line, and in vitro experiments (see in vitro table)	1 breeders X 4 litters X 5 pups=**20***
B6.129S4 dab1-1 (null)	Establish congenic line on C57BL/6	1 breeder pair X 4 littersX 5 pups=**20***
Balb.dab1 null	On this background homozygous mutants are viable past 3 weeks.	1 breeders X 4 litters X 5 pups=**20***
B6.dab1-5Fki	Establish congenic line on C57BL/6	1 breeder pair X 4 littersX 5 pups=**20***
B6.dab1-Wtki	Establish congenic line on C57BL/6	1 breeder pair X 4 littersX 5 pups=**20***
B6.129S4. dab1-4Fki	Establish congenic line on C57BL/6	1 breeder pair X 4 littersX 5 pups=**20***
B6.129S4. dab1-232Fki	Establish congenic line on C57BL/6	1 breeder pair X 4 littersX 5 pups=**20***
B6.Cg-TgN(Thy1-YFP)	crossed into B6.129S4dab1-1, B6.129S4.dab1-4Fki B6.129S4.dab1-232Fki lines	a) 3 pairs(YFP het. with dab1 mutant het.) X 6 pupsX 2 litters=**36** mice (9 correct double het genotype. b) 3 pairs (double het. from abovewith respective dab1 het. mutant) X 6 pups X4=**72** mice (desired animal heterozygous for YFP (0.5 and homozygous for dab1 mutation 0.25=0.125 or 9 mice controls are also derived from this cross.)
B6.129S4-Meox2(CreSor)	To maintain the colony to maintain mice to be bred with knock-in animals	1 breeder pair X 4 littersX 5 pups=**20***

	maintain mice to be bred with knock-in animals	1 breeder pair X 4 littersX 5 pups=**20***
B6.CAM KIICre	To maintain the colony to maintain mice to be bred with knock-in animals	1 breeder pair X 4 littersX 5 pups=**20***
B6.SJL-TgN(ACTFLPe)	To maintain the colony to maintain mice to be bred with knock-in animals	1 breeder pair X 4 littersX 5 pups=**20***

*(50% of mice correct genotype but since genotyping is done after weaning all mice are counted)

Animal Import/Export Guidelines

Animal Import/Export

NINDS / NIDCD

IMPORT/EXPORT

Importation and Exportation of animals is a potentially complicated process requiring transmission appropriate health information and coordination of several individuals at both the donor and recipi institutions. International shipments carry additional potential obstacles such as language barriers a import permits required by customs officials. International shipments frequently require greater involvement by the investigator.

There is a formal NIH policy governing the importation of rodents and rodent products which can l at: http://oacu.od.nih.gov/NIHpolicy/3043-1.pdf

Transferring animals between NIH locations

Transferring animals between NIH facilities is complicated by the organizational structure of the N Some facilities are operated by individual institutes, and some are operated by the Veterinary Reso Program (VRP). Various animal holding facilities house animals of different health statuses, and th facility has its own criteria for entry of new animals. However, transfers within NIH are less compl than external transfers in that they do not require an NIH importation application nor approval by t wide rodent import officer.

To initiate an import from another NIH location, send a written request to your institute's import co This request should specify:

- both the donor and recipient investigators, their institutes, their Animal Study Proposal numb the other facility's veterinary contact. Contact information, including telephone and fax num well as email addresses for all of these individuals should be provided.
- the species, strain, number, gender, age and immune competence of the animals to be shippe
- the current housing location and intended destination (building and room numbers) of the an
- how the animals may be easily identified for examination and packing by facility personnel,
- the Central Accounting Number that will be used to defray the costs for the transfer.

After submission of the request, the veterinarians communicate the appropriate health information evaluate whether quarantine and/or further testing is necessary. Animal facility personnel coordina transportation, and the investigator is notified when to expect the transfer to occur.

Importing animals from outside NIH

To initiate an importation from outside the NIH the investigator must complete and submit (fax) a Import Application (http://forms.cit.nih.gov/adobe/animals/NH2369_1.PDF) to your institute's imp contact. Some of the information requested on this form, such as the building and room number wh animals are housed, as well as the veterinary contact information, will need to be obtained from yo collaborator.

After submission of the request, the veterinarians communicate the appropriate health information evaluate whether quarantine and/or further testing is necessary. Animal facility personnel coordina transportation, and the investigator is notified when to expect the transfer to occur.

Exporting animals outside of NIH

Exports of animals outside the NIH are coordinated by the NIH shipping department. A number of documents must be completed to execute the transaction, including a health certificate, a shipping request, a written request for the services of the NIH shipping department and a Transfer Assuranc Agreement. For international shipments, a commercial invoice is also required.

To initiate an export, a written request should be submitted to your institute's export contact. This r should specify:

- the donating investigator, his/her Animal Study Proposal number and contact information, in telephone and fax numbers as well as email address
- the recipient investigator, their institution, and their veterinary and shipping points of contact Contact information for all of these individuals should be provided.
- the species, strain, number, gender, age and immune competence of the animals to be shippe
- the current housing location (building and room numbers) of the animals
- how the animals may be easily identified for examination and packing by facility personnel,
- the Central Accounting Number that will be used to defray the costs for the transfer.

Additionally, the investigator must submit a signed Transfer Assurance Agreement, a Shipping Cra Request Form, and a Request for Shipment Form to your institute's export contact. If you have que regarding completion of these forms, please call your institute's export contact.

After submission of these materials, the veterinarians communicate the appropriate health informat evaluate whether quarantine and/or further testing is necessary. Your institute's export contact com and submits the required documents to the NIH shipping department. The shipping department con and returns documents authorizing the shipment to the AHCS. Animal facility personnel coordinat transportation, and the investigator and the recipient institution are notified when to expect the tran occur.

Import/Export Contacts

NINDS
Dr. James O'Malley
301-402-0068
301-402-5424 fax
omalleyj@ninds.nih.gov

NIDCD
Dr. James McGehee
301-402-0223
301-402-0541 fax
mcgeheej@nidcd.nih.gov

Animal Ordering Guidelines

http://ahcs.ninds.nih.gov/ordering.html

Animal Ordering

NINDS / NIDCD

Investigators with an approved ASP can order from an approved vendor through the Delpro System, using Centralized Animal Procurement System (CAPS) or with the NIH Centralized Animal Order Request (NIH 79-3). Approved sources of rodent or rodent products have a contract established with the Veterinary Resources Program (VRP), ORS or a comparable contract with other programs within NIH to supply genetically-defined, specific pathogen-free animals to NIH investigators. These contracts require barrier production practices, genetic management and monitoring, microbiologic standards and health surveillance, and regular site visits to ensure the availability of high-quality animals suitable for NIH research.

What is CAPS and how do I get access? Instructions [MS Word Document]

Approved vendors:

Charles River Labs

Harlan Sprague Dawley

Jackson Laboratory

Taconic Farms

Covance

DCT Division of Cancer Treatment, Frederick

National Institute on Aging — aged rodents

National Institute of Health Animal Center (NIHAC) — dogs, cats, pigs, nonhuman primates.

Unapproved vendors:

Animal orders from unapproved vendors must be placed on a Requisition Worksheet (NIH 1861-3) and the requisition must be sent to the AHCS for approval **BEFORE** the order can be placed.

ANIMAL ORDERING

Small animals may only be purchased for use in research after an animal study proposal (ASP) has been fully approved by the NINDS/NIDCD ACUC. To order animals, the principal investigator (or designee) completes an animal order in the Centralized Animal Procurement System (CAPS). CAPS is a subsystem of the NIH Administrative Database (ADB) and is used for placing, approving and receiving small animal orders as well as for paying vendors and billing ICs. Principal investigators or designees should request access to CAPS through their Administrative Officer (AO). The AO will need to have 2 accounts set up for each investigator. The NIH Center for Information Technology (CIT) account gives the user access to the NIH mainframe. The ADB account provides access to CAPS.

1. A CIT Account. This account gives the user access to the NIH IBM 3270 mainframe. Each user will have an account number and user initials
2. IMSGATE/ADB SETUP. Gives the user access to the CAPS system within the ADB. The AO will set up the user to have IC functions.

All animal orders must be placed in CAPS 5 BUSINESS DAYS IN ADVANCE OF THE DATE REQUESTED FOR DELIVERY. Vendor schedules for NIH delivery vary:

Approved Vendors*	**Delivery days**
Charles Rivers Labs	Tuesday, Wednesday, Thursday
Taconic	Tuesday, Thursday
Jackson Labs	Thursday only
Harlan Sprague Dawley	Mon, Tue, Wed, Thurs, Fri
DCT	Mon, Tue, Wed, Thurs, Fri

*Approved Vendors are those listed on the Veterinary Resources Program (VRP) indefinite delivery contract.

Animal orders placed in CAPS less than 5 business days in advance are considered “emergency orders” and a 50% surcharge is added to the cost of the order. Standing orders may be set up in the CAPS system, but must be renewed at the change of the fiscal year.

After the order has been placed in the CAPS system, the order is forwarded to the Institutional Approving Official (IAO). The IAO ensures that the person ordering the animals, the number and type of animals requested, and the delivery point are consistent with the ASP. The IAO then forwards the order to the facility in which the animals will be housed. The Facility Manager ensures that space and resources are available. Once the IAO and Facility Manager have approved the Animal Order, the order is forwarded to the VRP small animal ordering section who actually place the order with the vendor. Animal orders from NIH approved vendors MUST be placed through the CAPS systems. It is a violation of Federal Procurement Regulations for investigators to place orders directly with vendors.

For animals ordered from nonapproved vendors the PI must submit a completed NIH 79-3 (NIH Centralized Animal Order Request) (available at: K:\APPS\NINDS 2K FormFlow Shortcuts\NIH_Forms) to the AHCS office (fax 402-5424) for IAO and facility approval. When the order has been signed by both the AHCS IAO and the facility manager of the requested holding location, the signed order will be faxed back to the investigator and then the signed order may be placed through the regular purchase office.

For information on importing or exporting animals please refer to information that can be found at http://ahcs.ninds.nih.gov/. For other questions, contact the Animal Health and Care Section (AHCS) 496-9354.

NIH Animal Transportation Guidelines

NIH Animal Transportation Guidelines

A. General

1. All methods of transporting NIH animals must provide for the health and welfare of the animals.

2. Transportation of animals shall be done in a direct and timely manner, avoiding public areas and areas primarily used by NIH employees and patients.

3. Animals shall not be transported with any other animal, substance or device that may be expected to be injurious to their health or welfare.

4. Care shall be exercised in handling enclosures used to transport live animals. They shall not be tossed, dropped, needlessly tilted, stacked in a manner which may reasonably be expected to result in their falling, or handled in any manner which may cause physical trauma or stress to the animals.

5. Temperature extremes are to be avoided when animals are transported and special precautions or postponements are required when temperatures are below 45 degrees Fahrenheit or above 85 degrees Fahrenheit and may jeopardize the welfare of the animals.

6. The Animal Welfare Regulations (AWRs) shall be followed in transporting regulated laboratory animals in intra or interstate commerce. However, the AWRs specific enclosure standards are not applicable for hand carrying rodents in containers between buildings on the NIH campus.

7. Transportation of animals must comply with applicable state and local laws and regulations.

8. It is essential that primary enclosures be used in the transportation of animals, and that they be escape proof, properly labeled, provide adequate ventilation, can be sanitized or disposed of and prevent the spread of pathogenic microorganisms, chemicals or radioactive materials where indicated. The enclosures should be opaque or shielded in such a way as to be nonstressful to the animals.

9. Cargo areas used in the transportation of animals shall be cleaned and decontaminated as necessary to prevent contamination of future animal deliveries.

10. Veterinary Resources Program (VRP) provides a central Animal Transportation Service (301-496-8184) for NIH with environmentally controlled trucks and trained drivers. It is available as needed for the delivery of all species of animals on the NIH campus and locally. Its use is required for transporting primates, farm animals, dogs and cats off the NIH reservation unless another acceptable method is justified and approved by the Institute or Center (IC) Veterinarian.

11. ICs may develop specific procedures for the transportation, receipt and shipment of animals if they have requirements that differ from these guidelines. The responsibility for development and approval of these specific IC procedures lies with the IC Scientific Director (SD), following recommendations of that IC's Animal Care and Use Committee (ACUC). A dated copy of the written guidelines shall be forwarded to the NIH Office of Animal Care and Use.

12. The IC Veterinarian (or other IC) Animal Transportation Coordinator as designated by the (IC SD) is responsible for oversight of these NIH (and/or IC) animal transportation guidelines, can grant exceptions when it is considered in the best interest of the animal(s), and is the contact person for information concerning the transportation, receipt and shipment of animals. Conflicts regarding animal transportation issues will be resolved by the IC ACUC or NIH ARAC.

B. Movement of animals within an NIH building

1. Occupants of the building should be protected from allergens of animal origin, microorganisms, chemicals, radioactive materials and escaped animals.

2. See Clinical Center (Bldg. 10) Research Animal Transportation Policy 614, dated 8/00.

C. Moving animals between buildings on the NIH Bethesda campus

Proper containment of animals transferred between buildings is essential. When practical the VRP Transportation Service should be used for the movement of dogs, cats, primates and farm animals. Transporting rodents by hand carrying them should be limited to travel in a direct and timely manner between buildings with the animals in escape proof enclosures and when all the general (Item A) preceding requirements are met. Rodents and rabbits should be transported in screened (filter-covered) enclosures (cages or disposal transport boxes). The interior of disposable transport boxes should be individually examined before direct disposal - such transport boxes should not be left in corridors for disposal. An immobilizing drug and physical containment system should be used when transporting primates between buildings unless the entire caging system can be relocated with the animals in place.

D. Transportation of animals between the NIH Animal Center (NIHAC) and the Bethesda campus

The VRP Transportation Service must be used for transporting animals between the NIH campus and NIHAC unless otherwise approved by the IC Veterinarian. VRP has a regularly scheduled daily animal delivery service between the NIHAC and Bethesda campus. An environmentally controlled truck delivers animals from the NIHAC to Bethesda in the morning and from Bethesda to NIHAC in the early afternoon. Arrangements can be made for scheduling animal deliveries by calling 301-496-8184. Special arrangements can also be made by calling VRP in advance.

E. Delivery of animals to locations outside NIH

1. **Health certificate** - For non-rodent animals being shipped from Maryland to another state or country, a United States Department of Agriculture/Animal Plant Health Inspection Service certificate of veterinary inspection is required. The appropriate form (i.e. interstate vs. international, species, appropriate, must be signed by a USDA accredited veterinarian within 30 days of shipment and accompany the animals.

2. **VRP Transportation** - VRP transportation is limited to Montgomery County, Baltimore, and regional transportation terminals.

3. **NIH Shipping Unit** - Call 301-496-5921 for commercial shipping information and complete NIH form 1884 to request animal shipments.

Air shipments of laboratory animals are made by having the NIH Shipping Unit book flights on commercial airlines and having the VRP Transportation Service deliver animals to the local airport prior to the flights. Arrangements must be made by the consignor to have the animals picked up by the consignee at the airport of destination.

The use of an airfreight company for door-to-door delivery of mice and rats only, can be arranged by the NIH Shipping Unit. This means can be used only when atmospheric temperatures are above 45 degrees Fahrenheit or below 85 degrees Fahrenheit, because the company may not have access to environmentally controlled vehicles or holding areas.

4. **Other** - Any other arrangements that are made for transporting animals outside of NIH must meet requirements of the AWRs and be approved by the IC Veterinarian. An Animal Transfer Agreement (see ARAC Guidelines) may be needed when transferring government-owned animals to a non-government research facility.

F. Transporting live or dead animals containing radioactive isotopes

1. Investigators planning to transport live animals containing radioactive materials from one location to another should contact the Radiation Safety Branch at 496-5774 for specific guidance, unless the two locations are within the same building.

2. A radiation safety protocol is required for the use of any amount of radioactive material in large animals (dogs, sheep, monkeys, etc.) and for the use of large amounts of radioactive material in small animals. Special transportation requirements must be addressed in such protocols.

3. Warning labels are required on enclosures used to transport live or dead animals that have been exposed to radioactive hazards. The specific hazard must be identified.

G. Transporting animals treated with human pathogens or carcinogenic material

1. NIH policy (PM 3040-2) requires that IC Animal Care and Use Committees review animal study proposals for research with animals, including work with biohazards or chemical hazards. The transportation of animals that are to be dosed at one location and moved to another needs to be particularly evaluated to assure that proper containment is used to minimize occupational exposure to persons involved with the

move, and to minimize environmental contamination. The Occupational Safety and Health Specialist (496-2346) shall be consulted.

2. Small laboratory animals that have been exposed to human pathogens or toxic/ carcinogenic substances and are actively shedding the hazardous material must be transported in closed systems. Transportation needs for larger animals so exposed will have to be evaluated on a case-by-case basis by the IC Veterinarian in consultation with the Occupational Safety and Health Specialist.

3. Warning labels are required on enclosures used to transport live or dead animals that have been exposed to chemical or biological hazards. The specific hazard must be identified.

4. Carcasses of contaminated animals must be handled according to the guidelines of the Environmental Protection Branch (496-3537) for handling as Medical Pathological Waste or for disposal as chemical waste. Contaminated animal carcasses that are being transported for pathological examination also need to be placed in double plastic bags (primary barrier) and then into a cardboard box (secondary barrier) and must be accompanied by a detailed history of the type and amount of hazardous material.

Approved - 10/1/88
Reapproved - 5/8/96
Revised - 2/10/99
Revised - 9/12/01

Transportation Guidelines for Building 49

Procedures for Transport of Live Animals Out of the Bldg 49 Central Animal Facility

The Central Animal Facility provides Procedure Room for Investigators to conduct experimental procedures on their animals. If for some reason animals must be transported out of the CAF to a lab or for some procedure, the animals may not return to the CAF. The animals MUST NOT be transported out of the CAF in facility cages.

In order to minimize the risk of contamination of the animal being transported and of other areas and animals within the CAF and the following procedures must be followed:

To transport animals within Building 49 or to locations outside the building –

1. Obtain a cardboards transport box from the procedure room on the floor where the animals are housed and a sheet of tilter paper. These boxes come in two sizes, small and large. If you are unable to locate a transport box, contact the Team Leader or the Evening Technician.

2. Fold the two short flaps on the bottom of the box in first and then the 2 long flaps of the box.

3. Tape the seam down the center of the box with tape. Then tape the two short side seams of the bottom of the box.

4. Cut two rectangles of the filter paper large enough to cover the three vents on either side of the box.

5. Tape the filter paper over the vents on either side of the box.

6. Take the box and the tape to the animal housing room and using proper micro-isolator technique, transfer the animals to the box from the cage.

7. Fold the short flaps in first and the fold the long flats over.

8. Tape the center seam. Tape the side seams so that all openings in the box are cover with either tape or filter paper.

9. Return the tape to the procedure room

10. Animals in transport boxes can leave the CAF either through the lobby door to the C-corridor or via Elevator #5. Elevator #5 provides access to the 4^{th} through the 6^{th} floors.

Alternative method of transporting animals within Building 49 ONLY

1. Obtain either a small or large ice cream container from the procedure room. These containers are only for transportation of single mice, neonatal rats and mice, and rats under 50gms.

2. Loosen the inner lid of the container with a pen or pair of scissors. DO NOT perforate the inner lid.

3. Take the container to the animal housing room and using proper micro-isolator technique, transfer the animals to the container from the cage.

4. Place the lid firmly on the container ensuring that no part of the animal with be caught under the lid.

5. Animals in ice cream containers can leave the CAF either through the lobby door to the C-corridor or via Elevator #5. Elevator #5 provides access to the 4^{th} through the 6^{th} floors.

Transportation Guidelines for Building 50

SOP6200	**NIAID/DIR Animal Care Program** Buildings: 50
Title: Outgoing Animals and Transferring Animals to Another ASP	**Date: February, 2002** **Supercedes Date:**

To minimize the risk of transferring pathogens between NIAID/DIR animal facilities, it is imperative that the movement of animals into and out of the facilities be monitored and controlled. The health status of animals must be the same as or better than the animals already in the facility into which they will be transferred. The facility supervisor must be informed when animals enter or leave a NIAID/DIR facility. He/she must know their source or destination and their eventual disposition before animals may enter or leave the facility. No animals may be maintained outside of the animal facility. Delivery destinations must be listed on the ASP or Animal Transfer Agreement. Because they pose a threat to the health of resident animals, removed animals may not return to the facility.

To initiate the movement of animals held in Building 50 to another location, the investigator submits an Animal Transfer Form (Attachment 6200A) to the technical staff or electronically to acb50techs@niaid.nih.gov. The supervisor in Building 50 contacts the supervisor in the destination facility to confirm space availability.

An Animal Transfer Form (Attachment 6200A) is sent from the supervisor in the facility of origin to the supervisor in the destination facility before transportation is arranged. Once transportation is arranged, the plan is recorded in the Outgoing Deliveries Log (Attachment 6200B). It is the responsibility of the facility supervisor to ensure that animals are packed and ready in time for the scheduled delivery. For scheduling purposes, it is requested that a minimum of 48 hours notice be given when requesting the transfer of animals.

For intra-NIAID movements that involve transfer of animals from one ASP to another, it is the responsibility of the supervisor of the receiving facility to notify the 14B South Administrative Staff, before transportation is arranged. The transfer request must contain the new protocol number.

Refer to SOP3005 and 3006 for information regarding bar coding and change log procedures.

Building 50 will only accept animals from sources other than approved vendors, if that source has maintained a one-year health history comparable to that of Building 50.

Transportation Guidelines for 5 Research Court

NIDCD/DIR/Animal Health & Care Section
Building: 5 Research Court (5RC)

Title: 5RC Animal Import & Export Procedures

Date: March, 2004

To minimize the risk of transferring pathogens between 5 Research Court and other animal facilities, it is imperative that the import and export of live or dead animals to the 5RC facility be monitored and controlled. The health status of animals imported into the 5RC main colony must be the same as or better than the animals already in the colony and according to facility policy. The 5RC Facility Veterinarian must approve all plans and transfers for the importation or export of live or dead animals involving the 5RC animal colony. He/she must know their source or destination and their eventual disposition before live or dead animals enter or leave the facility. In the case of live animal transfers between NIH Institutions, an NIDCD Rodent Transfer form must be completed. For scheduling purposes, it is requested that a minimum of 48 hours notice be given when requesting a live animal transfer. In the case of live animal exports to non-NIH institutions, a signed NIDCD Gift Letter and Animal Transfer Assurance Agreement must be completed.

NIH guidelines/policies concerning animal transport must always be followed. Only vehicles with climatic control can be used to transport live animals. Privately owned vehicles cannot be used to transport any live or dead animal.

To initiate any import or export of animals concerning the 5RC animal colony, please contact the Facility Veterinarian at (301) 402-0223.

Transportation Guidelines for VRP Managed Facilities (14 Complex, Poolesville)

SOP NO. 900 **Approved** *Charmaine Foltz, D.V.M.* **Date 01/01/04**
Acting Director, Division of Veterinary Resources Program, ORS

Date Issued 09/22/03

Date Revised N/A **Page 1**

TITLE	**:**	Transportation Requests
SCOPE	**:**	All personnel
RESPONSIBILITY:		Facility Manager, Transportation Personnel, Veterinarians
PURPOSE	**:**	Facilitation of timely transport of animals and to meet health and behavior requirements

1). General hours of business are between 7:00 AM and 4:00PM Mon. through Fri., excluding holidays. Service may be arranged outside of these hours upon request at least 3 days in advance. (Staff overtime costs will be assessed outside of these hours). Number (301) 496-8184.

2). Regular daily schedules have been established to transport large animals to and from the NIH Animal Center in Poolesville. Refer to this schedule when requesting transportation & planning procedures on the appropriate day for each species.

DAY	**MON**	**TUES**	**WED**	**THURS**	**FRI**
SPECIES	NHP	Ungulates, Cats	DOGS	NHP	Ungulates, Cats

3). It is imperative to coordinate arrangements in advance through facility managers, technicians, or veterinarians at both the pick-up and delivery sites. Determine which forms, if any are required and who will call the VRP Transportation Office to schedule the transport.

4). Veterinary emergencies will take precedence over all other requests. Other unusual circumstances may require special transport. These will be handled on a first come , first served basis, if resources are available.

5). Animal transportation requests are prioritized as follows:
   a). Animal emergency transports
   b). Time critical transport such as airport, surgery, NIMR/PET scans
   c). Standing regularly scheduled large animal transport
   d). Rodent and rabbit transport on campus

6). Determine availability of transportation staff and vehicles **before** scheduling procedures or making airline arrangements for a particular date.

7). Provide the following information to transportation:

   Name and phone number of investigator AND a contact person
   Date and time of requested transport
   Species and quantity of animals being shipped
   Pick-up and Delivery sites
   Special Requirements: Time critical, Radioactive, Biohazard etc.
   FOR AIR SHIPMENTS: Airway Bill #, Flight #, Airline and Airport

8). Whenever possible, request transportation late morning or early afternoon to avoid scheduling conflicts with regular large animal shipments or creating delays.

SOP NO. 902 **Approved** *Charmaine Foltz, D.V.M.* **Date 01/01/04**
Acting Director, Division of Veterinary Resources Program, ORS

Date Issued 9/15/89

Date Revised 7/22/02

TITLE	**:**	Transporting Animals Within NIH
SCOPE	**:**	NIH Intramural Animal Program
RESPONSIBILITY:		All VRP Personnel Transporting Laboratory Animals Within NIH
PURPOSE	**:**	To Transport Laboratory Animals According to Appropriate

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Note: Refer to the NIH Animal Transportation Guidelines

General Principles

1. All methods of transporting NIH animals must provide for the health and welfare of the animals. Care shall be exercised in handling enclosures used to transport live animals.

2. Transportation of animals shall be in a direct and timely manner, avoiding areas primarily used by NIH employees, patients and the public.

3. Animals shall not be transported with other animal species, substances or devices that may be injurious to their health or welfare.

4. Enclosures used to transport live animals must be properly labeled, disposable or easily cleaned and disinfected, strong enough to contain animals comfortably and securely to withstand the normal rigors of transportation. The interior shall be properly ventilated, have no sharp edges or protuberances, shall not allow any part of the animals body outside in a way that could result in injury to itself or others, and shall be large enough to allow for normal postural adjustments of the species being transported.

5. Provide food and a source of water when animals are expected to remain in the boxes for more than two hours.

    a. Provide enough food and water for twice the estimated delivery time.
    b. Provide the same type of food fed to animals prior to shipment.

6. Use a sufficient amount of the same type of bedding that is used in the facility to absorb animal waste if the transportation enclosure is not of the type with a raised or suspended floor.

7. Temperature extremes are to be avoided when animals are transported and special precautions or postponements are required when temperatures are below 45 degrees Fahrenheit or above 85 degrees Fahrenheit. Animals should not be exposed to extremes in noise, temperature, drafts, cold winds or direct sunlight.

8. Do not place animal transportation enclosures near steam pipes, radiators, stoves, other sources of heat or potentially toxic materials.

9. Vehicles used to transport animals must be dedicated, environmentally controlled, and easily cleaned and disinfected.

10. Animal transportation vehicle environmental controls shall be adjusted to maintain the temperature of the animal holding compartment between 45 and 85 degrees F during animal transport. At minimum, monitor the temperature of the animal holding compartment at loading, every two hours while in transit, and when off loading. If the environmental controls malfunction and the animal holding compartment cannot be maintained between 45 to 85 degrees F, contact the Animal Transportation Unit at (301) 496-8184 immediately. Another environmentally controlled vehicle shall be dispatched to meet and complete the animal transport.

11. The IC veterinarian in coordination with the VRP veterinary staff may develop more specific procedures for the transportation, receipt and shipment of animals as required.

Transporting Rodents and Rabbits - Specific Guidelines

1. Divide the primary transportation enclosure into compartments, as needed, when shipping rats, mice, cotton rats, hamsters, and pregnant rodents.

2. The minimum floor space provided for animals in shipping primary enclosures is specified in Table 1 of this SOP. Use the values on Table 1 to ensure adequate area for the animals.

3. Increase the floor space of the container in hot weather (>75^{o}F), by reducing the number of animals in each shipping container by 50%. If filter material is used to cover ventilation openings, the number of animals per box is further reduced beyond the reduction necessary during hot weather.

4. Transporting rodents and rabbits by hand carrying them in escape proof primary transport enclosures shall be limited to travel between buildings on the NIH reservation in a direct and timely manner.

5. Unless in filtered containers, rabbits and rodents shall not be transported in the same compartment of vehicles with other species. It is permissible, however, to transport one species in unfiltered containers if other species are housed in filtered containers.

Transporting Dogs and Cats - Specific Guidelines

1. Use sanitizable cages or disposable crates to transport dogs and cats. A solid, leak-proof bottom with litter or removable, leak-proof collection tray under a mesh floor may be used.

2. The transportation cage used shall be large enough to ensure that each animal has sufficient space to turn about freely in a standing position, to stand and sit erect, and to lie in a natural position.

Transporting Ungulates and Poultry - Specific Guidelines

1. Animals of the same species and maintained in compatible groups may be transported together. Animals that have not reached puberty shall not be transported in the same enclosure with adults other than their dams. Females in estrus shall not be transported in the same enclosure as any males.

   Enclosures shall provide sufficient space to allow each animal to make normal postural and social adjustment with adequate freedom of movement.

Transporting Primates - Specific Guidelines

1. Primates may be tranquilized and placed in a physical containment system or moved in their cage. For old world species, transportation enclosures have a wire, rod or slat floor with a litter pan below. New world species may be transported on direct litter flooring.

2. The transportation enclosure used shall be large enough to ensure that each animal has sufficient space to turn about freely in a stance whereby both feet and hands are on the floor and can sit in an upright position.

3. Transport only one primate per cage except for the following situations: a mother and her nursing infant, an established male-female pair (unless the female is in estrus), a pair of juveniles of the same species that have not reached puberty and are an established pair.

4. An immobilizing drug and physical containment system may be used for transport of primates between buildings or the entire caging system is relocated with the animals in place.

Transporting Animals Treated With Human Pathogens or Carcinogenic Materials - Specific Guidelines

1. Transport rodents and rabbits in closed systems such as a disposable transport box or disposable cage. Transportation of larger animals will be evaluated on a case by case basis by the IC Veterinarian in consultation with the Occupational Safety and Health Specialist.

2. Place warning labels on containers of animals. Identify the specific hazard on this label.

3. Place carcasses of dead animals in a plastic bag inside a cardboard box for transportation to the Laboratory Sciences Section. Attach a form NIH 2141, see SOP # 1116, to the box. Include a detailed history of the type and amount of hazardous material used.

Transporting Animals Treated With Radioactive Materials - Specific Guidelines

1. The registered radiation user or designee shall contact the Radiation Safety Branch before transporting live animals containing radioactive materials.

2. Review appropriate radiation safety protocol for special transportation requirements.

3. Driver must wear a current radiation safety badge during animal transport and submit used badges for evaluation to Safety for evaluation at the end of each month.

4. The registered radiation user or designee shall place warning labels on containers used to transport live or dead animals exposed to radioactive materials. Identify the specific isotope and radioactivity on this label. Line the bottom of the containers with absorbent papers.

5. Dispose of containers and papers as radioactive waste.

Animal Delivery - Specific Guidelines

1. All deliveries shall be to the building and room designated by the animal order or transport request except where deliveries are made to the individual in charge of a centralized building.

2. All animals shall be delivered to a representative of the requesting laboratory, or facility who is required to physically accept the animals and the responsibility for their care.

3. If no one is present at the designated delivery point to receive the animals, take the animals to the Animal Procurement and make immediate arrangements for appropriate alternate temporary housing in the Building 14 complex. receiving area. For animal deliveries within building 10, see Building 10 Research Animal Transportation Policy 2-17.

4. Facility veterinarians or their designees shall coordinate deliveries between NIH facilities with the approval of the IC Veterinarian.

Animal Transportation Vehicle Sanitation - Specific Guidelines

1. Clean vehicle animal holding compartment of any gross debris. Sanitize with TBQ or quatricide and rinse thoroughly with fresh water. (TBQ at 2 ounces per gallon dispensed in sprayer hose attachment or diluted in mop bucket and applied to surfaces with mop)

2. Sanitize vehicle animal holding compartments after transporting primates, carnivores, ungulates, and poultry. Sanitize vehicles used to transport rodents in filtered containers weekly, or more frequently as needed to maintain a high level of sanitation.

TABLE 1

Species	Minimum Floor Space per Animal (square inch)	Minimum Interior Height of Shipping Container (inch)
Mice		
Up to 5 weeks	3.0	4.0
5-8 weeks	4.5	
8-12 weeks	6.0	
Over 12 weeks	7.5	
Rats		
Up to 50 g	6.0	6.0
50-100 g	9.0	
100-150 g	12.0	
150-200 g	15.0	
200-300 g	20.0	
Over 300 g	25.0	
Hamsters		
Up to 60 g	6.5	5.0
60-80 g	8.0	
80-100 g	11.0	
Over 100 g	13.0	
Guinea Pigs		
Up to 350g	30.0	8.0
350-600 g	45.0	
Over 600 g	55.0	
Rabbits		
3-5 lbs.	90.0	12.0
6-8 lbs.	180.0	
9-11 lbs.	270.0	
Over 12 lbs.	360.0	

Transportation Guidelines for Bldg 10A to MIF and From MIF to Bldg 10A

POLICY FOR THE TRANSPORTATION OF ANIMALS FROM BUILDING 10A TO THE MOUSE IMAGING FACILITY AND RETURN TO 10A

1. This policy addresses only the reentry of mice and rats to the 10A facility following imaging in the Mouse Imaging Facility (MIF). In spite of the name, this facility can also be used to image rats and other small animals. Mice from other NIH animal facilities can not be housed in the 10A MIF quarantine area. The policy pertains to animals presently in the 10A facility that plan on being returned to the 10A facility, unless terminal procedures take place during the quarantine phase.

2. The transport of animals to and from the imaging facilities is the responsibility of the investigator and must be coordinated with the 10A staff. Transportation of these animals must comply with the applicable guidelines and policies; the animals must be kept from public view. Mice and rats are to be transported in microisolator cages. The cage and its filter must be banded together to prevent the escape of the animals in case of an accident. Food and water bottles should be removed.

3. Investigators are obligated to use every reasonable precaution to prevent their animals from contacting potential murine pathogens in the mouse imaging facility. This includes disinfection of surfaces and gloved hands with the disinfectant supplied by the facility and the use of disinfected restraint devices (disinfected by the facility). Keep in mind that other animals being imaged in this facility will come from facilities of varying pathogen status.

4. Investigators must notify the 10A facility (phone 402-0456) when animals will be leaving and returning so that facility personnel can meet them at the entry to the 10A facility. Returning animals are to be taken by the 10A staff to the B1 entry to the 10A facility off the building 10 D corridor. Animals may be returned to the 10A facility from 7:00 AM to 7:00 PM on weekdays only. The imaging facility can house mice overnight, but they must be removed from these holding rooms by 12:00 noon on Fridays and by the same hour on any day before a holiday. Arrangements to house mice in the MIF must be made in advance and approved by the MIF staff.

5. Upon receipt of animals being returned from imaging, facility personnel will place the cage(s) in a plastic bag and immediately take it to the quarantine room set aside for animals after imaging. The microisolator cages are removed from the plastic bag in the biosafety hood, the animals are observed and placed in a clean microisolator cage, the bottom of the new cage and the operator's gloved hands are sprayed with Clidox, and then the cage(s) are placed on the cage rack. The used cage(s) is placed into the plastic bag, the bag sprayed with Clidox, removed from the room, and the bag and cages are autoclaved.

6. During quarantine the following applies:

a. One sentinel cage of two immuno-competent animals will be set up for each group of returning animals. A group is all of the animals returned by one investigator for up to two weeks. In addition to the cage of sentinels, one easily identified immuno-competent female sentinel will be placed in each cage of imaged animals. Testing for a group starts when the last animal(s) return from imaging.

b. If a group of animals will be imaged several times, testing for these animals will start when the animals return after the last imaging. The investigator needs to communicate to the facility if animals will be serially imaged and when.

c. Dirty bedding from the cages will be placed in a cage used for sentinel animals every time the cages are changed.

d. During quarantine, moribund imaged animals will be submitted for a complete diagnostic work-up.

e. After 5 weeks of quarantine, all sentinel animals will be submitted for a complete screen.

f. If test results are negative for murine pathogens, the animals will then be returned to the using IC's cages. If the testing indicates that the animals have been exposed to murine pathogens that are not permitted in the 10A facility, the animals in that group must be removed from the 10A facility within 3 work days.

g. Animals that are returned after imaging that will not be returned to a holding room in 10A may be held a maximum of 6 weeks. The use of sentinels and the testing of theresearch animals is at the discretion of the facility veterinarian.

h. The cost of sentinels and testing of returning animals in quarantine will be charged to the investigator. During the quarantine period, only 10A animal care personnel will have access to the area, investigators can not manipulate or perform procedures on the animals during this time.

Additional Information:

A. Mice that are to be serially imaged may be housed in the mouse imaging facility during the week if advance arrangements have been approved by the MIF staff. These animals must be removed from the holding rooms of the mouse imaging facility by noon on Friday and by noon on any day preceding a holiday.

B. Dr. Davis will share health standards that are applied to the use of the mouse imaging facility with Dr. O'Brien as they are developed.

_____

Signature of Principal Investigator - Date

Policy Approved: June 27, 2002

Examples of Section D: Study Objectives

Examples of Study Objective Descriptions

Example 1

One of the most devastating complications associated with malignant brain tumors is brain swelling. Because the brain is contained in the skull, the brain cannot expand to accommodate increased fluid, and the pressure in the brain increases. This prevents normal functioning of the brain, and results in significant neurologic deficits and even death. The reason this happens is because the brain tumor cells alter the structure and function of the blood vessels in the brain around which the tumor cells grow. Normal blood vessels have a blood-brain barrier (BBB), a functional and structural entity which prevents access into the brain of many chemicals and fluid from the circulation. This allows the brain to remain in a very controlled environment, which is necessary given the delicate nature of the neurons and other CNS cell types.

However, in the presence of tumor cells, the BBB is reduced or destroyed at the location of the tumor. This allows too much fluid to cross into the brain, and thus cause swelling, known as brain tumor-associated edema. Even a small focal tumor can result in substantial fluid accumulation throughout the brain, leaving the patient in a neurologically compromised life-threatening state. Despite attempts at some new therapeutic approaches to the treatment of brain tumor patients, the only effective treatment for brain tumor-associated edema remains administration of high dose steroids, in particular dexamethasone, a synthetic corticosteroid often used at lower doses for more common problems such as allergic inflammation.

The requirement for use of such high doses is not trivial, because these doses exacerbate the side effects known to be associated with dexamethasone administration, including Cushings syndrome, diabetes, hypertension, ulcers, and immunosuppression. Because dexamethasone is a fat-soluble molecule, it was initially assumed that it should readily cross the BBB. Thus the observed requirement for high doses was thought to be the result of an altered steroid receptor in brain tumors (thus requiring an increased amount to be activated) or the result of some un-elucidated mechanism not involving the receptor. In fact, studies examining this issue have demonstrated that neither of these explanations is adequate. The receptor is normal and the effects on brain edema are receptor-mediated. The explanation for this discrepancy between the predicted and required dose necessary to treat this problem is the basic issue for study in this protocol. Understanding this may lead the way to different drugs which are more effective in treating this problem, but with fewer side effects; or to the inclusion of other drugs which allow the currently used steroids to work more effectively.

Example 2

Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare neurometabolic disease of GABA degradation. The human disorder has a wide range of symptomatology, including severe developmental delay and subsequent mental retardation, seizures, behavioral disturbances, and ataxia. This autosomal recessive disorder is suspected by the detection of 4-hydroxybutyric aciduria on urine organic testing in patients with the suspected clinical picture. As a result of SSADH deficiency, patients show elevated levels of gamma-aminobutyric acid (GABA), the predominant inhibitory neurotransmitter, and gamma-hydroxybutyrate (GHB), a metabolite of GABA. Our knowledge about this disease is limited and dependent upon isolated retrospective case reports. Long-term complications in patients include neurocognitive impairment, disproportionate language dysfunction, hypotonia, hyporeflexia, behavioral disturbances (hyperactive behavior, psychosis), and seizures, but the pathophysiology remains undefined. To explore the patho-physiologic mechanisms involved in human SSADH deficiency, and to investigate the potential development of pre-clinical therapeutics, Gibson et al. developed a murine model of this disorder using standard gene targeting methodology (Hogema et al 2001, Nature Genet., 29:212). The availability of this strain of mice, in which the gene encoding SSADH is disrupted, has promoted recent insights in GABAergic and glutamatergic systems, and has provided opportunities for novel treatment regimens that may be applicable to patients with this disease.

There is no standard therapy for SSADH deficiency. Patients are treated symptomatically, and a range of antiepileptic drugs (AEDs) have been used for seizures, with a variable level of success. The most commonly prescribed antiepileptic is vigabatrin, as this inhibits GABA-transaminase and thus the formation of succinic semialdehyde, the substrate for SSADH. Yet vigabatrin has met with limited success in clinical practice in these patients, and is furthermore complicated by the visual loss associated with retinal toxicity. In the animal model, generalized tonic-clonic seizures occur almost universally by 20 days of age, leading to death. Vigabatrin, phenobarbital, and phenytoin have been tested for their antiepileptic properties and survival effects, but results have been disappointing. A trial of taurine was used after the observation that the mice developed convulsions upon weaning, and taurine is of high concentration in murine breast milk. There was a modest prolongation of survival with this agent. In light of recent evidence by Gibson's lab (Gibson et al. 2002, J. Neurochem. 81:71-9) that there are not only regional elevations in GABA but also glutamate in temporal lobe tissue in mice with homozygous SSADH deficiency, we propose to study several AEDs with different mechanism of action in the animal model. In this proposal, we plan to administer lamotrigine (LMG), levetiracetam (LVT), topiramate (TPM), felbamate (FBM), valproic acid (VPA) and vigabatrin (VGB) to mice deficienct in SSADH. The rationale for selecting these AEDs, planned doses, etc. will be presented in Section F.

NINDS/NIDCD Animal Care and Use Committee (ACUC) Guidelines for Performance of Multiple Major Survival Surgical Procedures

NINDS/NIDCD Animal Care and Use Committee (ACUC) Guidelines
for
Performance of Multiple Major Survival Surgical Procedures

REFERENCES
AWA Section 13(a)(3)(D,E) and 9 CFR, Part 2, Section 2.31 (d)(1)(x)

USDA/APHIS Policy #14: *Major Survival Surgery Single vs. Multiple Procedures*, April 14, 1997.

PURPOSE
The NINDS/NIDCD ACUC recognizes there are instances when investigators have scientific justification to perform multiple survival surgical procedures. The need may be based on experimental design or may be a result of developments encountered in ongoing research. All survival surgical procedures must be performed aseptically in accordance with the *Guide for the Care and Use of Laboratory Animals* and the ARAC's "Guidelines for Survival Surgical Procedures."

PROCEDURE
Multiple survival surgical procedures are approved providing the following conditions are met:

1. There must be a need that precludes the use of separate animals. Primarily the reason must be scientific in nature but on occasion, may be based on species conservation. Major survival procedures must be included in the animal study proposal and reviewed by the NINDS/NIDCD ACUC. Convenience or monetary savings are not adequate justification.

2. The procedures must not compromise the animals' ability to perform normal body functions (eating, drinking, righting itself, etc.).

3. The proper use of postoperative analgesics and antibiotics and proper postoperative care must be described and provided.

4. The animal must be allowed sufficient time to fully recover from the effects of the previous surgical procedure -- generally a minimum of seven (7) days. Measurable physiological parameters relevant to the species (e.g. EKG, hemogram, PVC, serum chemistry for large animals; body weight, normal activity etc for rodents.) must be within normal limits unless variations are the results of an approved surgical manipulation.

5. The Principal Investigator must include in Section G. (Survival Surgery), paragraph 6, the length of time (number of days) the animal is allowed to recover after each surgical procedure. Unless scientifically justified, research procedures should not be performed until the animal has fully recovered from surgery.

These guidelines are not intended to prohibit the use of multiple surgical procedures when required to provide state-of-the-art veterinary medical care.

Revised and Approved: 12/1998, 5/2001, 9/2003

NINDS/NIDCD Animal Care and Use Committee (ACUC) Policy for Rodent Tail Snip and Altricial Pup Identification

NINDS/NIDCD Animal Care and Use Committee (ACUC) Policy for Rodent Tail Snip and Altricial Pup Identification

REFERENCE

ARAC *Guidelines for the Euthanasia of Mouse and Rat Fetuses and Neonates* (Revised 3/27/02)

NIH *Guidelines for the Genotyping of Rodents* (Approved 6/12/02)

ARAC *Recommendation on Toe Clipping of Animals* (Revised 9/12/01)

PURPOSE

The NINDS/NIDCD ACUC recognizes the need to specify a policy on procedures for rodent tail snip and use of toe-clip as a means of identification in rodents.

PROCEDURE

I.

Rats – Newborn to P12
Mice – Newborn to P14

Anesthesia is not required for tail snipping procedures if animals are less than P14 (mice) or P12 (rat) days of age. However, we encourage the use of isoflurane for this procedure.

II.

Animals older than P12 (mice) or P14 (rats) should be anesthetized for tail snip procedures. We recommend and encourage the use of isoflurane, which is an effective and safe inhalant anesthesia. Pups 14 days of age or older are anesthetized in a pre-charged clear chamber and anesthesia safely maintained at a flow of 2.0% isoflurane with 2 LPM O_2. Animals are placed in the chamber and allowed to achieve effective anesthesia within 1 to 2 minutes of exposure to isoflurane. Once anesthetized, animals are individually removed, and tails snipped. Pups are usually fully recovered within 3 to 5 minutes. If the procedure is not completed before the pup begins to recover, the animal can be safely returned to the chamber for additional anesthetic. Pups can be placed individually or as a group into the induction chamber. Litters of pups have been safely maintained at 2.0% isoflurane for a length of time with no added risk of death due to anesthetic overdose. Alternatively, the tail can be disinfected with 70% ethanol and allowed to dry, followed by an application of ethyl chloride spray or other suitable topical anesthetic as recommended by a veterinarian. Once pups are fully recovered (in a warmed cage) they can be returned to the parent cage. Deaths secondary to anesthesia or maternal rejection have not occurred, in our experience.

For pups 21 days or older, general anesthesia must be used.

For the initial “snip” the length removed may not exceed 0.2 cm (2 mm) of tail. If an additional sample is needed later, the total amount of tail removed must not exceed 0.5 cm (5 mm) of tail. Tail stumps are cauterized with silver nitrate sticks or "quick stop."

III

If the pup's ears are too small to be tagged or punched, alternative identification methods must be used. The ACUC will consider a request for toe clipping as an acceptable method of identification in altricial (< 7 days of age) neonates if the investigator clearly states in the protocol that toe clipping will be performed in conjunction with tail snip procedures prior to P7 (rats and mice).

Toe clip is prohibited for neonates P7 or older. If ear tagging is required and the animals are 3 weeks old or older, we recommend ear tagging or ear punches performed under anesthesia.

Information for NINDS/NIDCD Animal Study Proposals (ASPs) Involving Use of the NMR Center (NMR Form)

Information for NINDS/NIDCD Animal Study Proposals Involving Use of the NMR Center

In order to provide required veterinary support and to ensure the health and well-being of animals are maintained during procedures in the NMR Center, the following information is requested. Use of this form is required for NINDS/NIDCD Animal Study Proposals, and it is suggested that all users of the NMR Center use it to provide their IACUC with specific information concerning use of the NMR Center. Although some information requested below is also on the *NMR CENTER ANIMAL PROCEDURE REQUEST FORM*, it is necessary to provide the information so individuals reviewing your Animal Study Proposal can plan for the services you require.

- **Submitted:** ☐ With initial protocol ☐ As addendum to protocol # _____
- **ASP Title:** _____

- **Magnet to be used:** _____

- **Length of procedure** (approximate)**:** _____

- **Will animal arrive anesthetized?** ☐ Yes ☐ No. All animals except rodents must arrive to the Center under general anesthesia.

- **Anesthesia to be used:**

  - For transport to the NMR Center

    Agent(s): _____

    _____

    Route(s) of administration: _____

  - For procedures in the NMR Center

    Agent(s): _____

    _____

    Route(s) of administration: _____

    Procedure for bolus administration (if chemical anesthesia is used): _____

    _____

    _____

* Please Note: Ketamine alone is not sufficient for scanning procedures.

Monitoring of the Animal During the Procedure: All animals must be monitored for depth of anesthesia. We highly recommend that, at a minimum, respiration, heart rate/pulse, and body temperature are monitored. Please give a brief explanation as to how the animal(s) will be monitored for level of anesthesia and general well-being during the procedure, and who will do the monitoring. A record of anesthesia and anesthetic monitoring must be maintained and filed in the appropriate Animal Study Proposal file in the NMR Center upon completion of the procedure.

_____

_____

_____

NOTE: This form must be submitted with your Animal Study Proposal to the IACUC. The NMR Center protocol form must be submitted to the NMR Center Protocol Committee along with a copy of your IACUC-approved Animal Study Proposal.

PI Signature: _____ **Date:** _____

Example Intervention and Endpoints Table

EXAMPLE INTERVENTION AND ENDPOINT TABLE

Sign/Symptom	Intervention Assessments	Treatment Actions/Endpoints*
Awakening from survival surgery	Check for body temperature; check for dehydration; check for response to physical stimulation	1) Maintain animal warm with heated pad; inject fluids, if needed; 2) Euthanize if unresponsive; 3) If animal appears normal/healthy, move back to holding facility in clean cage with bedding, water and food. Initiate yellow post-operative card, advise veterinarian.
Animal not active (somnolent) but otherwise appears healthy	Weigh the animal and compare to pre-surgical wt	Monitor by the veterinarian
Weight loss, rough hair coat not as active as normal (e.g. burrowing or not moving unless stimulated)	Fluids SQ and possibly analgesics	1) Weigh animal daily; provide high caloric Jell-O supplement 2) Give fluids, if needed 3) Give analgesics, if needed. If animal does not respond within 3 days (e.g. gaining wt, active, alert) then euthanize
Head Tilt, Circling	Weigh; check for dehydration	1) PVC or skin tent to determine if dehydrated 2) Compare weights 3) Follow veterinarian's advice after assessment– may include limited treatment and if no response, euthanize
Inability to right itself; hypothermic	None	Euthanize
Evidence of infection at incision site: redness or darkening, swelling, hot to touch, exudates	Culture the wound; clean the incision site	1) Place on antibiotic treatment per veterinary instructions; 2) Daily wound care (clean) 3) Consider use in acute study or euthanasia depending on severity of infection and the professional judgment of the veterinarian
Evidence of inflammation at injection site	Observe inflamed area for necrosis for 24 hours	1) Apply topical antibiotic twice daily and possibly SQ analgesic. 2) Should the area of necrosis increase in size or depth, consider use in acute study or euthanasia depending on severity of tissue injury and the professional judgement of the veterinarian.

*All treatments will be initiated by the veterinary staff, who will maintain contact with the PI/P.O.C listed on the Disposition Instruction Form. No antibiotic treatments or euthanasia will be done without an attempt to consult with the PI/P.O.C. The veterinarian will take the appropriate action in an emergency if no response from the PI/P.O.C is received within a half hour after attempt to notification was made. The PI or the responsible investigator will have filled out a yellow surgical card with date and time of surgery, anesthesia used, etc.

ARAC Guidelines for Survival Blood Sampling in Animals

GUIDELINES FOR SURVIVAL BLEEDING OF MICE AND RATS

These Guidelines have been developed to assist investigators and institutional Animal Care and Use Committees (ACUC) in their choice and application of survival rodent bleeding techniques. The guidelines are based on peer-reviewed publications as well as on data and experience accumulated at NIH. It is the responsibility of both the investigator and ACUC to use techniques and procedures which result in the least pain and distress to the animal, while adequately addressing the needs of the experimental design. **Training and experience of the phlebotomist in the chosen procedure are of paramount importance. Training opportunities and resources, including access to experienced investigators and veterinarians, must be made available to new personnel.** Each ACUC should establish lines of accountability to oversee the training of its personnel. The procedures utilized must be reviewed and approved by the ACUC prior to their implementation.

Factors to consider in choosing the blood withdrawal technique appropriate for the purpose at hand include, but are not limited to:

- The species to be bled.
- The size of the animal to be bled.
- The type of the sample required (eg. serum, whole cells, etc.).
- The quality of the sample required (sterility, tissue fluid contamination, etc.).
- The quantity of blood required.
- The frequency of sampling.
- Health status of the animal being bled.
- The training and experience of the phlebotomist.

Both the quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal. The approximate blood volume of a mouse is 72 ml/kg $\pm$ 8 ml and 64 ml/kg $\pm$ 6 ml for the rat (e.g., 1.5 ml for a 20 gr mouse and 13 ml for a 200 gr rat). In general, no more than 10% of the animal's blood volume should be removed at one sampling. Volumes greater than 10% should be justified in the ASP and appropriate fluid replacement considered. Suggested recovery periods vs. blood sample size are provided in Table I.

The following guidelines refer to the most frequently used survival sampling sites: a) Tail; b) Retro-orbital; c) Saphenous and d) Jugular. Blood withdrawal by cardiac puncture is considered a terminal procedure and should be performed only after ensuring that the animal is under deep anesthesia. Issues that should guide the choice of survival blood collection route(s) is listed below, and an abbreviated summary is provided as Table II.

Lateral Tail Vein or Ventral/Dorsal Artery Sampling:

- Can be used in both rats and mice by cannulating the blood vessel or by nicking it superficially perpendicular to the tail.
- Obtainable volume: Mouse - small to medium
  Rat - medium
- Sample collection using a needle minimizes contamination of the sample, but is more difficult to perform in the mouse.
- Sample collection by nicking the vessel is easily performed in both species, but produces a sample of variable quality that may be contaminated with tissue and skin products.
- Sample quality decreases with prolonged bleeding times and “milking” of the tail.
- Repeated collection possible.
- Relatively non-traumatic.
- Routinely done without anesthesia, although effective restraint is required.
- In most cases warming the tail with the aid of a heat lamp or warm compresses will increase obtainable blood volume.
- In general, arterial sampling produces larger volumes and is faster, but special care must be taken to ensure adequate hemostasis.
- For a one-time collection of a very small sample, i.e., a single drop of blood, snipping of no more than the distal 1 mm of the tail can be a viable alternative.

Retro-orbital Sampling:

- Can be used in both rats and mice (though usually not a method of choice in the rat) by penetrating the retro-orbital plexus/sinus with a glass capillary.
- The NIH ARAC has determined that in the hands of a skilled operator retro-orbital bleeding is a humane procedure that produces minimal and transient pain/distress.
- Rapid – large number of mice can be bled within a short period of time.
- Obtainable volume: medium to large.
- Good sample quality. Potential contamination with topical anesthetic, if used, should be taken into account.
- Not amenable to frequent repeated sampling from the same orbit (10 days to 2 weeks recommended between successive bleeds).
- In the hands of an unskilled operator, retro-orbital sampling has a greater potential than other blood collection routes to result in complications.
- The presence of a plexus rather than sinus in the rat can lead to greater orbital tissue damage than in the mouse.
- Retro-orbital bleeding can be conducted in awake mice. A topical ophthalmic anesthetic should be applied prior to the procedure. Alternatively, systemic anesthesia should be considered if compatible with experimental design.
- Due to restraint issues retro-orbital sampling in the rat should be conducted under general anesthesia.
- In both mice and rats, care must be taken to ensure adequate hemostasis following the procedure.

Saphenous/Lateral Tarsal Sampling:

- Can be used in both rats and mice by piercing the saphenous vein with a needle.
- Obtainable blood volumes: small to medium.
- Repeated/serial sampling is possible.
- Variable sample quality.
- The procedure is customarily done on an awake animal, but effective restraint is required.
- Relatively low throughput technique compared to retro-orbital sampling due to time required for adequate site preparation (shaving).
- Requires more hands-on training than tail or retro-orbital sampling to reliably withdraw more than a minimal amount of blood. Prolonged restraint and site preparation time can result in increased animal distress when handling an awake animal. Temporary favoring of the limb may be noted following the procedure.

Jugular Sampling:

- Limited to the rat.
- Obtainable blood volumes: medium to large.
- High sample quality.
- Jugular sampling can be conducted without anesthesia, although the use of anesthesia greatly facilitates the procedure.
- Does not lend itself to repeated serial sampling.

Table I: Blood Sampling Volumes and Recovery Periods*

Single Sampling		**Multiple Sampling**	
% Circulatory Blood Volume Removed	**Approximate Recovery Period**	**% Circulatory Blood Volume Removed In 24 Hr.**	**Approximate Recovery Period**
7.5%	1 Week	7.5%	1 Week
10%	2 Weeks	10-15%	2 Weeks
15%*	4 Weeks	20%*	4 Weeks

*With higher withdrawal volumes, additional monitoring (e.g. hematocrit, hemoglobin) and appropriate fluid replacement should be considered.

Table II: Summary of Blood Sampling Techniques

Route	General anesthesia required	Speed and efficiency		Sample quality		Repeated sampling	Relative volumes obtainable	Potential for complications	Species	Comments
		Mouse	Rat	Mouse	Rat					
Tail Vein or Artery	no	++ Vein +++ Artery	+++ Vein +++ Artery	± to ++[1]	++ to +++	yes	small (vein) medium (artery)	low	Rat, Mouse	Repeatable, simple, variable sample quality
Retro-orbital	Mouse – no[2] Rat- yes	+++	++	+++	++	difficult	medium to large	moderate to high	Rat, Mouse	Rapid, potential for complications
Saphenous/ Lateral Tarsal	no	++	++	++	++	yes	small to medium	low	Rat, Mouse	Not as rapid as other techniques, low potential for tissue damage
Jugular	Recom-mended		+ /++		+++	difficult	large	low	Rat	Limited application, poor for repeated sampling

[1] Depending on method and amount of manipulation

[2] Topical anesthesia recommended

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Guide for Survival Bleeding of Mice and Rats

Guidelines for Survival Blood Sampling in Animals

These guidelines have been developed to assist investigators in their choice and application of survival blood sampling techniques while writing and conducting an Animal Study Proposal (ASP).

Factors for Consideration and Required Detailed ASP Information

a. Species - Various species have various estimated percentages of Total Blood Volume (TBV) based on Total Body Weight (TBW)

Examples:	Mouse	TBV estimated mean of 7.2% of the TBW
	Rabbit	TBV estimated mean of 5.6% of the TBW
	Rat	TBV estimated mean of 6.4% of the TBW
	Rhesus macaque	TBV estimated mean of 5.6% of the TBW
	Beagle Dog	TBV estimated mean of 8.5% of the TBW
	Guinea Pig	TBV estimated mean of 7.5% of the TBW
	Minipig	TBV estimated mean of 6.5% of the TBW
	Horse	TBV estimated mean of 8.5% of the TBW

b. Animal Size (Weight) - Various animals within a species have various TBWs which in turn effects TBV. TBV is calculated using the TBW (in kilograms or grams) multiplied by the species dependent TBV percentage with a straight conversion of weight to volume (ex. kilograms = liters and grams = milliliters).

c. Restraint or Sedation Techniques - ASP must include a full and detailed technical description and justification for restraint or sedation.

d. Sample Desired (serum, whole cells, plasma, etc.) - ASP must include a full and detailed justification of why you need to conduct blood sampling.

e. Sample Quality (i.e., level of required sterility and techniques dictated by that level)

f. Sampling Site - ASP must include a full and detailed description and listing of all possible techniques and sites, respectively, that would be appropriate for the species and your research goals. It is always best to list as many alternatives as possible, especially when you consider "Murphy's Law."

Blood Sampling Site Examples:

Dorsal/Ventral Tail or Central Pinna Artery

Retrobulbar venous plexus - ASP must include a procedural description of this technique that includes topical anesthesia and alternating eyes for multiple draws

Jugular, Saphenous, Lateral Tarsal, Sub-Lingual, Cephalic,
Femoral, Marginal Ear, and Lateral Tail Veins

Cardiocentesis, Cranial Vena Cava, and Drip Collection by Tail Tip Amputation (≤2mm)

g. Animal's Health Status - Consideration of the animal's health status should always be made prior to blood sampling to assess the procedure's possibly affect on the animal.

h. Phlebotomist's Experience & Expertise - ASP must include specific identity of qualified individuals with a description of their expertise.

i*. Sample Quantity & Sampling Frequency* - ASP must include a full and detailed description, justification, and time line for single, multiple, or serial blood sampling.

Generally, no more than 15% of the animal's blood volume should be removed at one time. Rapid sampling rates or sampling of greater than 15% of the TBV in one iteration could possibly result in circulatory shock resulting in adverse affects to the animal. If 10 -15% of the TBV is removed in one sampling, the same volume of warm physiologic saline or Lactated Ringer's should be given back to the animal intravenously at an appropriate rate or via clysis by subcutaneous injection.

Blood Sampling Volume Limits and Recovery Periods

Single Sampling		Multiple Sampling	
% circulatory blood volume removed	**Approximate recovery period**	**% circulatory blood volume removed in24 h**	**Approximate recovery period**
7.5%	**1 week**	**7.5%**	**1 week**
10%	**2 weeks**	**10-15%**	**2 weeks**
15%	**4 weeks**	**20%**	**3 weeks**

Blood Sampling Calculations

General Equation

TBW X %TBW for species TBV X Desired % of TBV for Draw X one milliliter/gram (or one liter/kilogram) = Draw Volume in mls or liters

1. 10% TBV Single Sampling 20 grams X .072 (7.2%) X .1 (10%) X milliliter / gram = .144 mls or
   Mouse, 20 grams TBW 144 microliters

2. 7.5% TBV Single Sampling 250 grams X .064 (6.4%) X .075 (7.5%) X milliliter / gram = 1.2 mls
   Rat, 250 grams TBW or 1200 microliters

3. 5% TBV Single Sampling 4 kgs X .056 (5.6%) X .05 (5%) X liter / kilogram = .0112 liters or
   Rabbit, 4 kilograms TBW 11.2 mls

4. 15% TBV Single Sampling 8 kgs X .056 (5.6%) X .15 (15%) X liter / kilogram = .0672 liters or
   Rhesus macaque 67.2 mls
   8 kilograms (kgs) TBW

5. 10% TBV Single Sampling 500 kgs X .085 (8.5%) X .10 (10%) X liter / kilogram = 4.25 liters
   Horse, 500 kgs TBW

An Example of a Drug Table

EXAMPLE
Table of Experimental Drugs

Name	Concentration	Dose & Volume	Route of Administration		Side-effects
			Site	Route	
USPIO (Iron Oxide Particles)	~30-1000nm Diameter	10-400umol of iron/kg body weight; 1ml or less for IV < 1ul forstereotaxic	Rear Leg or Brain	IV or Stereotaxic injection	None
Gadolinium-DPTA	.2 mM/ml	0.05-0.2mmol/kg body weight; < 1ml for IV or IA < 1ul forstereotaxic	Rear Leg or Brain	IV, IA, or Stereotaxic	None
Manganese Chloride	30-120mM in isotonic pH-buffered Saline	Rats-up to 885umoles/kg body weight Mice-442umoles/kg body weight; Rats-2ml/hour Mice-0.25ml/hour	Rear Leg or Abdomen	IV or IP	hypothermia, dehydration
Manganese Chloride	3.9mmol/ml in isotonic pH-buffered Saline	1-4ul/mouse	Naris	Pipetted into the naris or at the base of the whiskers	Possible bleeding caused by tubing
Manganese Chloride	10mM in isotonic pH-buffered Saline	1ul. into specific areas of the brain; Max. dose is 0.4umoles/kg body weight	Brain- thalamus, ventricles, or cortex	Stereotaxic	None
Mannitol	25% Solution	5mg/kg body weight; single dose	Neck	IA	None-terminal experiment
Cereport (RNP-7)	9 ug/ml	9.0ug/kg body weight; single dose	Rear Leg	IV	None-terminal experiment
D-Amphetamine Sulfate	10 mg/ml	20mg/kg body weight	Rear Leg	IV	None-terminal experiment
Endothelial Growth Factor (EGF)	360 ng/ml	20-360ng	Brain ventricles	Stereotaxic	None
Nitric-Oxide Synthase (NOS) Inhibitors	Vary according to type; 1 mM solution in artificial CSF	Doses vary according to type used and mode of application; 30 mg/kg body weight, 40 mg/kg body weight	Rear Leg, Abdomen, Brain-exposed cortex	IP, IV, or Topical over exposed cortex of brain	None

Common Drugs for Use in Animals

Common Drugs For Use in Animals

The following pages provide tables of drugs commonly used at the National Institutes of Health (NIH) for pre-anesthesia, anesthesia, analgesia, sedation, tranquilization, and restraint of laboratory animal species.

The dosage recommendations and other data presented on the following pages are based upon current data in the literature and the professional judgement of veterinarians.

Proper drug doses may vary greatly depending on species, strain, sex, age, physiologic status of the animal, and the level of anesthesia/analgesia desired.

Although these lists provide a ready source of information on drug doses, individuals should not use these drugs without prior experience.

Your institute or animal facility veterinarians are available for consultation and additional information.

The page facing each table provides species specific information.

Controlled drugs are identified by a "C." The Roman numeral classifies the drug into one of the five established schedules of controlled substances (e.g., sodium pentobarbital, C-II).

Abbreviations:
IV = intravenous
IM = intramuscular
IP = intraperitoneal
SC = subcutaneous
PO = per os, oral
IH = inhalation
qXh = every X hours

SPECIES INFORMATION

MOUSE (*Mus musculus*)

Physiologic parameters:

Body temperature = 36.5-38.0ºC
Heart rate = 325-780/min
Respiratory rate = 94-163/min
Tidal volume = 0.09-0.23 ml

The use of chloroform as an anesthetic agent is discouraged. Chloroform can cause renal tubular calcification and/or necrosis, particularly in male mice; DBA/2 strain most susceptible.

Avertin is made by mixing equal amounts of tribromyl ethyl alcohol and tertiary amyl alcohol (2.5% dilution). If Avertin is improperly prepared or stored in the light, it will break down into dibromoacetic acid and hydrobromic acid which can be lethal in 24 hours. **Freshly mixed solutions are strongly recommended for safe use.** The solution can be kept as long as 4 months if it is stored in the dark at 4 degrees C. The solution should be tested to ensure that it has a pH >5.

* The therapeutic dose for carbon dioxide is close to the lethal dose; very short acting. Concurrent administration of 10-50% O_2 is recommended.

** Best for minor surgery procedures only.

MOUSE (*Mus musculus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.02-0.05 mg/kg	SC, IM
Diazepam, C-IV (Valium®)	5 mg/kg	IP
Ketamine, C-III (Ketaset®, Vetalar®)	22-44 mg/kg	IM
Carbon dioxide* + 10-50% O_2	To effect	IH
Anesthesia		
Sodium Pentobarbital, C-II	50-90 mg/kg	IP
Ketamine**, C-III	50-200 mg/kg	IP
Avertin (Tribromoethanol)	125-250 mg/kg 0.02 ml/g (1.2% solution)	IP
Ketamine/Xylazine:		
Ketamine	80-100 mg/kg Ketamine	IM, IP
Xylazine	5-7 mg/kg Xylazine	IM, IP
Isoflurane	To effect	IH
Analgesia		
Morphine, C-II	5-10 mg/kg q2-4h	SC IP
Butorphanol tartrate (Torbugesic®), C-IV	2.5-5 mg/kg q1-2h	SC
Buprenorphine, C-V	0.05-0.1 mg/kg q8h	SC IP
Oxymorphone, C-II	0.15 mg/kg q4h	IM
Ketoprofen	5 mg/kg q24h	SC

SPECIES INFORMATION

RAT (*Rattus norvegicus*)

Physiologic parameters:

Body temperature = 35.9-37.5°C
Heart rate = 250-450/min
Respiratory rate = 70-115/min
Tidal volume = 0.6-2.0 ml

Male rats and animals receiving low calorie diets require higher doses of barbiturates.

Avertin has been reported to cause ileus in rats

The therapeutic dose for carbon dioxide is close to the lethal dose; very short acting. Concurrent administration of 10-50% O_2 is recommended.

The reversal agent, yohimbine, is only effective when xylazine or medetomidine has been used.

* The projected duration of action for an analgesic is an approximation because the nature of the procedure and the level of pain that is experienced affect it.

RAT (*Rattus norvegicus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.04-0.1 mg/kg	SC
Diazepam, C-IV (Valium®)	5-15 mg/kg	SC
Ketamine, C-III (Ketaset®, Vetalar®)	22-50 mg/kg	IM
Carbon dioxide + 10-50% O_2	To effect	IH
Anesthesia		
Sodium Pentobarbital, C-II	30-60 mg/kg	IV IP
Ketamine, C-III (10 mg/ml solution)	50-100 mg/kg IP	IM
Ketamine/Xylazine:		
ketamine	60-80 mg/kg	IM
xylazine	5-7 mg/kg	IM
Halothane (Fluothane®)	To effect	IH
Isoflurane	To effect	IH
Carbon dioxide	To effect	IH
Telazol®, C-III	20-40 mg/kg	IP
	20 mg/kg	IM
Ketamine/Medetomidine		
ketamine	60-75 mg/kg	IP
Medetomidine (Domitor®)	0.25-0.5 mg/kg	SC
Analgesia*		
Morphine, C-II	1.5-3 mg/kg q2-4h	SC
Butorphanol tartrate, C-IV (Torbugesic®)	2.5-5 mg/kg q1-2h	SC
Ketoprofen	5 mg/kg q12h	SC
Buprenorphine, C-V	0.01-0.05 mg/kg	SC
Reversal Agents		
Yohimbine (reverses xylazine)	1-2 mg/kg	IM IP

SPECIES INFORMATION

HAMSTER (*Mesocricetus auratus*)

Physiologic parameters:

Body temperature = 37-38$_{o}$C
Heart rate = 250-500/min
Respiratory rate = 35-135/min
Tidal volume = 0.6-1.4 ml

Syrian or golden hamster is very resistant to morphine - no sedation or hypnotic effects.

Syrian or golden hamster has an increased tolerance to pentobarbital.

HAMSTER (*Mesocricetus auratus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	.1 mg/kg	IP IM SC
Ketamine, C-III (Ketaset®, Vetalar®)	20-60 mg/kg	IM
Diazepam	5 mg/kg	IP, IM
Anesthesia		
Sodium Pentobarbital, C-II	100-200 mg/kg	IP
Ketamine/Xylazine:		
Xylazine	7-10 mg/kg	IP IM
Ketamine	80 mg/kg	IP
Telazol®, C-III	20-80 mg/kg	IP IM
Isoflurane	To effect	IH
Analgesia		
Buprenorphine, C-V	0.05-0.1 mg/kg q8-12h	SC IM
Butorphanol tartrate, C-IV (Torbugesic®)	1-5 mg/kg q2-4h	SC IM

SPECIES INFORMATION

GUINEA PIG (*Cavia porcellus*)

Physiologic parameters:

Body temperature = 37.2-39.5°C
Heart rate = 230-380/min
Respiratory rate = 42-104/min
Tidal volume = 2.3-5.3 ml/kg

Large cecum can act as reservoir for anesthetics. Depending on drug solubility, the cecum can alter the pharmacologic effect.

Induction of anesthesia using volatile anesthetics (e.g., halothane and isoflurane) should be done with caution due to initial breath holding when animals are first exposed to irritating gas vapors.

Repeated exposure to halothane can cause hepatotoxicity. Isoflurane is a safer inhalant anesthetics to use.

Self mutilation has been reported in guinea pigs after ketamine administration.

GUINEA PIG (*Cavia porcellus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.05 mg/kg	SC
Diazepam, C-IV (Valium®)	2.5-5.0 mg/kg	IP IM
Acetylpromazine	5-10 mg/kg	M SC IV
Ketamine, C-III (Ketaset®, Vetalar®)	22-30 mg/kg	IM
Anesthesia		
Sodium Pentobarbital, C-II	15-40 mg/kg	IP
Sodium Thiopental, C-III	20 mg/kg	IV
Ketamine, C-III	40-50 mg/kg	IM
Ketamine/Xylazine:		
Xylazine	5 mg/kg	SC
Ketamine	30-40 mg/kg	SC
Isoflurane	To effect	IH
Analgesia		
Buprenorphine, C-V	.01-0.05 mg/kg q8-12h	SC
Morphine, C-II	10 mg/kg q2-4h	SC IM
Butorphanol tartrate, C-IV (Torbugesic®)	0.25-0.4 mg/kg	IV SC
Reversal Agent:		
Atipemazole (Antisedan®)	1 mg/kg	IM IV SC IP

SPECIES INFORMATION

CHINCILLA *(Chinchilla laniger)*

Physiologic parameters:

Body temperature = 38-39 C
Heart rate = 100-150 min
Respiratory rate = 40-80 min

Chinchillas are extremely susceptible to stress, all surgical procedures should be undertaken with caution. Premedication should be utilized for all surgeries.

CHINCILLA *(Chinchilla laniger)*

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.5 mg/kg	IM
Acepromazine	0.5 mg/kg	IM
Valium	3.0-5.0 mg/kg	IM
Anesthesia		
Ketamine/Acepromazine:		
Acepromazine	0.5 mg/kg	IM
Ketamine	40 mg/kg	IM
Ketamine/Xylazine:		
Ketamine	40 mg/kg	IM
Xylazine	5 mg/kg	IM
Ketamine/Valium		
Valium	1.0-2.0 mg/kg	IM
Ketamine	20-40 mg/kg	IM
Pentobarbital	30 mg/kg	IV
	40 mg/kg	IP
Isoflurane	To effect	IH
Analgesia		
Consult your veterinarian		

SPECIES INFORMATION

Ground Squirrel **(to be completed)**

SPECIES INFORMATION

RABBIT (*Oryctolagus cuniculus*)

Physiologic parameters:

Body temperature = 38-39.6°C
Heart rate = 130-325/min
Respiratory rate = 32-60/min
Tidal volume = 4-6 ml/kg

Many rabbits have serum atropinesterase which causes reduced response to atropine. Glycopyrrolate, another anticholinergic, can be used instead of atropine.

Unique hypnotism or immobilization reflex has been observed in rabbits in the absence of drug use.

Large cecum can act as reservoir for anesthetics. Depending on drug solubility, the cecum can alter the pharmacologic effect.

Induction of anesthesia using volatile anesthetics (e.g., halothane and isoflurane) should be done with caution due to initial breath holding when animals are first exposed to irritating gas vapors.

Give IV injections via marginal ear veins.

Self mutilation has been reported in rabbits after IM ketamine administration. Dilution of ketamine with saline will limit this side effect.

RABBIT (*Oryctolagus cuniculus*)

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.04-0.5 mg/kg	SC IM
Ketamine, C-III (Ketaset®, Vetalar®)	15-50 mg/kg	IM
Acetylpromazine (Acepromazine)	1.0-10 mg/kg	IM SC IV
Ketamine/Acetylpromazine (10:1)	15-50 mg/kg	IM
Diazepam, C-IV (Valium®)	5-10 mg/kg	IV IM
Glycopyrrolate	0.005-0.011 mg/kg	IM
Butorphanol & Acepromazine		
Butorphanol tartrate, C-IV (Torbugesic®)	1 mg/kg	SC
Acetylpromazine	1 mg/kg	SC
Anesthesia		
Sodium Pentobarbital, C-II (3% solution given slowly to effect)	15-40 mg/kg	IV
Ketamine/Xylazine/Acepromazine:		
Xylazine	5-10 mg/kg	IM
Ketamine, C-III	35-50 mg/kg	IM
Acepromazine	0.75 mg/kg	IM
Ketamine/Midazolam		
Ketamine, C-III	25 mg/kg	IM
Midazolam, C-IV	1 mg/kg	IM
Ketamine/Diazepam		
Ketamine, C-III	15-50 mg/kg	IM
Diazepam, C-IV	5-10 mg/kg	IM
Ketamine/Acepromazine/Butorphanol		
Ketamine, C-III	35 mg/kg	SC
Acepromazine	0.75 mg/kg	SC
Butorphanol tartrate, C-IV (Torbugesic®)	0.1 mg/kg	SC
Isoflurane	To effect	IH
Analgesia		
Morphine, C-II	2-5 mg/kg q2-4h	SC IM
Buprenorphine, C-V	0.02-0.1 mg/kg q8-12h	SC
Butorphanol tartrate, C-IV (Torbugesic®)	0.1-1.5 mg/kg q4h	IV
	1.0-7.5 mg/kg q4h	IM SC
Flunixin meglumine (Banamine®)	1.1 mg/kg q12h	IM SC
Carprofen	1.5 mg/kg q12h	PO
Ketoprofen	3 mg/kg q12h	IM
Reversal Agents		
Yohimbine (reverses xylazine)	0.2 mg/kg	IV
Doxapram (all anesthetics)	5-10 mg/kg	IM, IV, IP

SPECIES INFORMATION

NONHUMAN PRIMATES

Physiologic parameters:

	Rhesus	Baboon
Body temperature	37-39°C	39°C
Heart rate	120-180/min	150/min
Respiratory rate	32-50/min	35/min
Tidal volume	21 ml	50 ml

The dosage and frequency of administration of all analgesic agents must be tailored to the animal, procedure, and magnitude of pain present. Combinations of narcotics and non-steroidal agents are commonly used. Consult your veterinarian for specific recommendations.

* Pre-medication with Atropine or Glycopyrrolate is suggested to avoid bradycardia and cardiac arrhythmias with these agents.

** Poor analgesia. Adequate for superficial procedures only!

NONHUMAN PRIMATES

Drug indication and Drugs	Dosage and Route of Administration	
Restraint/Preanesthesia		
Atropine	0.02-0.05 mg/kg	IM SC
Glycopyrrolate	0.005-0.01mg/kg	IM SC
Diazepam, C-IV (Valium®)	0.5-1.0 mg/kg	IM
Xylazine	0.5-2.0 mg/kg	IM
Anesthesia		
Sodium Pentobarbital, C-II	20-30 mg/kg	IV
Sodium Thiopental, C-III (2.5%)	15-20 mg/kg	IV
Ketamine/Xylazine*:		
Ketamine, C-III	7-10 mg/kg	IM
Xylazine (Rompun®)	0.25-2.0 mg/kg	IM
Ketamine/Diazepam**:		
Ketamine, C-III	5 mg/kg	IV
Diazepam, C-IV (Valium®)	1 mg/kg	IV
Ketamine/Midazolam**:		
Ketamine, C-III	15 mg/kg	IV
Midazolam, C-IV	0.5-0.15 mg/kg	IV
Telazol®, C-III	4.0-6.0 mg/kg	IM
Halothane (Fluothane®)	To effect	IH
Isoflurane	To effect	IH
Analgesia		
Morphine, C-II	1-2 mg/kg q4h	IM SC
Oxymorphone, C-II	0.15 mg/kg q4-6h	IM
Buprenorphine, C-V	0.01-0.03 mg/kg q8-12h	IM SC
Acetylsalicytic Acid (Aspirin)	10-20 mg/kg q6h	PO
Acetaminophen	10 mg/kg q8h	PO
Flunixin meglumine (Banamine®)	0.5 mg/kg daily	IM
Butorphanol tartrate, C-IV (Torbugesic®)	0.025 mg/kg q3-6h	IM
Naproxen	10 mg/kg q12h	PO
Ketorolac	15-30 mg/kg	IM
Reversal Agents		
Yohimbine (reverses xylazine)	0.05 mg/kg	IV
Naloxone (reverses opioids)	.1-0.2 mg/kg as needed	IV

AMPHIBIANS

Anesthesia

Amphibians must be kept moist over their entire bodies during anesthesia and recovery. Care must be taken that they do not become immersed, as this will result in drowning.

Tricaine (MS 222) -ethyl m-amino benzoate methanesulfonate (tricaine methane sulfonate) Should be buffered to neutral pH before use. MS222 must be disposed as chemical waste.

Immerse in water with agent added:
1:2000 to 1:1000 for adults (i.e.,5-10mg of tricaine in 1000 ml water)
1:3000 to 1:5000 for larvae

Induction in 5-20 minutes; maintain by moist cloth contact with MS 222 solution.

Recovery - keep at 22-26$_{o}$C; takes 3-6 hours; keep moist.

Benzocaine 100 mg/1000 ml water

Halothane/Isoflurane - 5% in anesthetic chamber; maintain at 3%.

Sodium Pentobarbital- 60 mg/kg; inject into dorsal lymph sac.

Analgesia

Chlorpromazine	32 mg/kg; inject into dorsal lymph sac
Chlordiazepoxide	90 mg/kg; inject into dorsal lymph sac
Buprenorphine, C-V	14 mg/kg; inject into dorsal lymph sac
Diphenhydramine	51 mg/kg; inject into dorsal lymph sac

FISH

Because fish breathe through gills rather than lungs, anesthesia must be delivered through an aquatic medium. Most fish induced by adding the anesthetic agent to the tank water. It is important to have two separate tanks; one for anesthesia and one for recovery. Water for anesthesia should be well-aerated to provide adequate oxygen and minimize the stress of induction. Food should be withheld for several hours prior to induction.

Tricaine (MS 222) -ethyl m-amino benzoate methanesulfonate (tricaine methane sulfonate)
Should be buffered to neutral pH before use. MS222 must be disposed as chemical waste.

Immerse in water with agent added; doses vary according to species:
1:20,000 (50 mg/liter) for tranquilization
1:10,000 (100 mg/liter) for surgical anesthesia

Induction occurs within 3 minutes, recovery takes 10-15 minutes after removal.

Benzocaine 20-30 mg/1000 ml water for tranquilization
50 mg/1000 ml water for surgical anesthesia

Etomidate is an analog of propoxate and provides sedation only. It should not be used for procedures requiring surgical anesthesia.

0.05 -0.5 mg/1000 ml for tranquilization during transportation
2-4 mg/1000 ml for sedation

Listing of NINDS/NIDCD Consulting Veterinarians

NINDS Programs and Branches Basic Neuroscience Program (BNP)		
Abbreviation	**Lab/Branch/Section/Unit Name**	**Consulting Veterinarian**
▪ LCNSS	Lab of Central Nervous System	NA
▪ LDN	Lab of Developmental Neurogenetics	Les Sekut, DVM
▪ LFMI	Lab Functional and Molecular Imaging	Andrea Barnes, DVM
▪ LMB	Lab of Molecular Biology	James O'Malley, DVM
▪ LMCN	Lab of Molecular and Cellular Neurobiology	Les Sekut, DVM
▪ LMMN	Lab of Molecular Medicine and Neuroscience	Judith Davis
▪ LNLC	Lab of Neural Control	Les Sekut, DVM
▪ LN	Lab of Neurobiology	James O'Malley, DVM
▪ LNC	Lab of Neurochemistry	James O'Malley, DVM
▪ LNP	Lab of Neurophysiology	Crystal Thomas, DVM
▪ CDNS	Cellular & Developmental Neurobiology Section	Crystal Thomas, DVM
▪ DNPU	Developmental Neural Plasticity Unit	James O'Malley
▪ DNA	DNA Facility	NA
▪ GPCR	G-Protein Coupled Receptors Section	Crystal Thomas, DVM
▪ ISHF	Insitu Hybridization Facility	James O'Malley, DVM
▪ MNU	Molecular Neurophysiology Unit	NA
▪ MPBU	Molecular Physiology & Biophysics Unit	Andrea Barnes, DVM
▪ MPS	Molecular Plasticity Section	NA
▪ MTBU	Membrane Transport Biophysics Unit	Crystal Thomas, DVM
▪ NCU	Neural Circuit Unit	Andrea Barnes, DVM
▪ NDS	Neural Development Section	Les Sekut, DVM
▪ NSU	Neil Shneider Unit	Les Sekut, DVM
▪ NTS	Neurotoxicology Section	NA
▪ RBU	Receptor Biology Unit	Crystal Thomas, DVM
▪ SFU	Synaptic Function Unit	Les Sekut, DVM
▪ SPU	Synaptic Physiology Unit	Crystal Thomas, DVM
▪ STU	Synaptic Transmission Unit	Andrea Barnes, DVM

NINDS Programs and Branches

Clinical Neuroscience Program (CNP)

Abbreviation	Lab/Branch/Section/Unit Name		Consulting Veterinarian
▪ BFSB	Biostatistics Branch		NA
▪ CES	Clinical Epilepsy Section		Andrea Barnes, DVM
▪ CNB	Clinical Neuroscience Branch		NA
▪ CNCS	Clinical Neurocardiology Section		Andrea Barnes, DVM
▪ CNSS	Cognitive Neuroscience Section		NA
▪ CNU	Cellular Neurology Unit		Crystal Thomas, DVM
▪ DMNB	Developmental & Metabolic Neurology Branch		Crystal Thomas, DVM
▪ ERS	Epilepsy Research Section		Andrea Barnes, DVM
▪ ETB	Experimental Therapeutics Branch	NHP	James O'Malley, DVM
		Rodents	Andrea Barnes, DVM
▪ MNB	Medical Neurology Branch		Les Sekut, DVM
▪ MNPS	Molecular Neurpharmacology Section		Crystal Thomas, DVM
▪ NGB	Neurogenetic Branch		Crystal Thomas, DVM
▪ NB	Neuroimaging Branch		NA
▪ NIB	Neuroimmunology Branch		Andrea Barnes, DVM
▪ NMDS	Neuromuscular Diseases Section		NA
▪ NPPS	Neurophysiological Pharmacology Section		Andrea Barnes, DVM
▪ SB	Stroke Branch		Les Sekut, DVM
▪ SNB	Surgical Neurology Branch	NHP	James O'Malley, DVM
		Rodents	Les Sekut, DVM

NIDCD Laboratories, Branches, and Sections Dr. Robert Wenthold, Scientific Director		
Abbreviation	**Laboratory/Branch/Section/Unit Name**	**Consulting Veterinarian ***
LMB	Laboratory of Molecular Biology	Andrea Barnes, DVM
LMG	Laboratory of Molecular Genetics	James O'Malley, DVM
LN	Laboratory of Neurochemistry	James O'Malley, DVM
	Branches	
HNSB	Head and Neck Surgery Branch	Les Sekut, DVM
SLB	Speech and Language Branch	James O'Malley, DVM
	Sections	
AHCS	Animal Health and Care Section	Les Sekut, DVM
GCRS	G-Coupled Receptors Section	Andrea Barnes, DVM
SAM	Section on Auditory Mechanics	Crystal Thomas, DVM
SB	Section on Biophysics	James O'Malley, DVM
SDN	Section on Developmental Neurosciences	Andrea Barnes, DVM
SSCB	Section on Structural Cell Biology	Crystal Thomas, DVM
SN	Section on Neurogenetics	Andrea Barnes, DVM
SSCRD	Sensory Cell Regeneration and Development	Les Sekut, DVM
VRS	Vaccine Research Section	Les Sekut, DVM

*Dr. McGehee reviews all NIDCD protocols.

Aseptic Surgical Techniques Training Instructions

http://ahcs.ninds.nih.gov/acuc/a_training.html

ACUC Animal Study Proposal

NINDS / NIDCD

Aseptic Training Instructions

Please follow the instructions below in the order in which they are listed. It is important to watch the video before proceeding to the test! Please note -- there is a classroom portion of training to complete after you finish the online training.

1. Watch the Aseptic Training Video. QuickTime or Windows Media Player version
2. Take the online test. Take test
3. After completing the online test, call the ACUC coordinator at 301-496-9354 to schedule the didactic portion of your training.

Report technical problems with the online test to nicholsb@ninds.nih.gov

Column E Explanation Form

COLUMN E Explanation Form

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study:

3. Species (common name) of animals used in this study:

4. Explain the procedure producing pain and/or distress, including reason (s) for species selected:

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Information below will NOT be forwarded to USDA as part of the Annual Report

IC _____ ASP Number _____ ASP Title _____

Signature of Principal Investigator

Endpoints in Animal Study Proposals

ENDPOINTS IN ANIMAL STUDY PROPOSALS

Introduction

Experimental studies may involve procedures that cause clinical symptoms or morbidity in animals. The Animal Care and Use Committee must consider the selection of the most appropriate endpoint(s). This requires careful consideration of the scientific requirements of the study, the expected and possible adverse effects the research animals may experience (pain, distress, illness, etc.), the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects. The effective use of endpoints requires that properly qualified individuals perform both general and study specific observations of the research animals at appropriate time points. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting the research objectives. Such endpoints are preferable to death or moribundity as endpoints since they minimize pain and distress. Efforts must be made to minimize pain and distress experienced by animals used in research.

Morbidity

Animal Study Proposals that include morbidity as an endpoint or that include animal procedures that have the potential to cause adverse sequella should address the following:

1. Criteria that establish when the endpoint has been reached.
    A. There are several examples in the literature that might be considered, including:
        1. Evaluation of five aspects of an animal's condition as described by Morton and Griffiths. These are body weight, physical appearance, measurable clinical signs, unprovoked behavior and response to external stimuli.
        2. Clinical observations used in cancer research and toxicological studies as described by Montgomery. Parameters include changes in general appearance, skin and hair, eyes, nose, mouth and head, respiration, urine, feces and locomotion (Table 1).
    B. The clinical signs, depending on severity and duration, that may constitute an endpoint include, but are not limited to:

        Rapid weight loss.
        Diarrhea, if debilitating.
        Progressive dermatitis.
        Rough hair coat, hunched posture, lethargy or persistent recumbency.
        Coughing, labored breathing, nasal discharge.
        Jaundice and/or anemia.
        Neurological signs.

Bleeding from any orifice.
Self-induced trauma.
Any condition interfering with eating or drinking (e.g. difficulty with ambulation).
Excessive or prolonged hyperthermia or hypothermia.

C. Additional signs in neoplasia studies that may constitute an endpoint include, but are not limited to:
    1. A tumor burden greater than 10% bw, and in an adult mouse, a mean tumor diameter exceeding 20 mm or in an adult rat, a mean tumor diameter exceeding 40 mm. Formulas for calculating tumor size can be found in the literature (see tumor size ref.).
    2. Tumors that ulcerate, become necrotic or infected.
D. Any animal found unexpectedly to be moribund, cachectic, or unable to obtain food or water.

2. A plan for monitoring the animals both before and after a change in any of the above aspects, providing care if appropriate, and increasing the level of monitoring. Monitoring or clinical care on weekends and holidays may require involvement of the investigative staff to supplement that provided by the animal care and veterinary staff.
3. Identification of personnel responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and persons responsible for euthanasia. Checklists/ score sheets may be helpful in ensuring appropriate observations are made, consistently interpreted, and properly documented.

Death or Moribundity

While it is preferable to use the earliest endpoints compatible with the scientific requirements of each study, there are studies that require moribundity or mortality as an endpoint. The moribund condition is defined as a clinically irreversible condition leading inevitably to death. In these studies, animals are permitted to die or become moribund, as a result of experimental procedures. In some cases, pain relieving measures are not used because such measures may compromise the experimental integrity of the study. Examples of research proposals that may have death or moribundity as an endpoint include: infectious disease studies, drug and toxicity studies, and cancer research. The following guidelines are suggested to assist the IC Animal Care and Use Committees in reviewing proposals with death or moribundity as endpoints.

Animal Study Proposals utilizing death or moribundity as an endpoint should contain the following information:

1. The scientific rationale for death or moribundity as an endpoint, including:
    A. What alternatives were considered, why morbidity as an endpoint cannot be used, and how alternatives will be used whenever possible.
    B. Why pain relieving measures cannot be utilized.

C. Number of animals to be used and why this is the minimal number of animals required.
D. Whether animals will be euthanized when moribund and if not, what information is to be gained in the interval between moribundity and death.

2. A plan for the following animal care and monitoring procedures:
   A. Animals involved in experiments that may lead to moribundity or death will be monitored daily by personnel experienced in recognizing signs of morbidity (illness, injury, or abnormal behavior) for at least the following: abnormal posture, rough hair coat, head tucked into abdomen, exudate around eyes and/ or nose, skin lesions, or abnormal breathing, difficulty with ambulation, decreased food or water intake, or self mutilation.
   B. The frequency of observation will be increased when animals exhibit the above or other signs of moribundity. Monitoring on weekends and holidays may require involvement of the investigative staff to supplement that provided by the animal care and veterinary staff. Designated personnel, including a veterinarian, should be notified as soon as animals show signs of disease. An assessment of the animals' condition should be made as soon as possible and a plan of action established.
   C. Consideration will be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely. Dead animals must be promptly removed.
   D. Written records will be kept of monitoring.

Approved by ARAC 10/9/96
Reapproved - 2/10/99
Revised by ARAC 3/8/00

General endpoint references:

Canadian Council on Animal Care (1998), *guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing*. Ottawa, Canada.

Hendriksen and Morton, ed. (1998), *Humane Endpoints in Animal Experiments for Biomedical Research*. Proceedings of the International Conference, 22-25 November 1998, Zeist, The Netherlands. Laboratory Animals Ltd, by Royal Society of Medicine Press Limited, London, England.

Institute for Laboratory Animal Research Journal (2000), *Humane Endpoints for Animals Used in Biomedical Research and Testing*. 41: No. 2

Morton and Griffiths (1985), Veterinary Record 116:431-43.

Montgomery (1990), Cancer Bulletin 42:230-237.

Toth (1997), Contemporary Topics 36:44-48.

Stokes (1999) *Humane Endpoints in Animal Experiments for Laboratory Animals Used in Toxicity Testing* Proceedings of the 3rd World Congress on Alternatives and Animal use in the Life Sciences, 31 August - 2 September 1999, Bologana, Italy.

United Kingdom Co-ordinating Committee on Cancer Research (1997), *UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia*, 2nd ed. London, England.

Tumor size references:

Bullard et al. (1981), J. Neuropath. Exp. Neurol. 40:410-427.
Tomayko and Reynolds (1989), Cancer Chemother. Pharmacal. 24:148-154.
Sung et al. (1993), Cancer Research 53: 2092-2099.
Welch et al. (1994), Oncogene 9: 255-262.
Hamm (1995), Contemporary Topics 34:69-71.

Approved by ARAC - 10/9/96
Reapproved - 2/10/99
Revised and approved by ARAC 3/8/00

Table 1.
Selected Clinical Observations Used in Cancer Research and Toxicological Studies

Parameter	What to look for
General Appearance	Dehydration, decreased body weight, missing anatomy, abnormal posture, hypothermia, fractured appendage, swelling, tissue masses, prolapsed, paraphimosis
Skin and Fur	Discoloration, urine stain, pallor, redness, cyanosis, icterus, wound, sore, abscess, ulcer, alopecia, ruffled fur
Eyes	Exophthalmos, microphthalmia, ptosis, reddened eye, lacrimation, discharge opacity
Nose, Mouth, and Head	Head tilted, nasal discharge, malocclusion, salivation
Respiration	Sneezing, dyspnea, tachypnea, rales
Urine	Discoloration, blood in urine, polyuria, anuria
Feces	Discoloration, blood in the feces, softness/diarrhea
Locomotor	Hyperactivity, hyperactivity, coma, ataxia, circling, muscle, tremors

Montgomery, C.A. Jr. (1990), *Cancer Bulletin* 42:230-237
and appeared in AWIC Newsletter, Spring 1995 6:4

ARAC Guidelines

Guidelines for Euthanasia of Rodents Using Carbon Dioxide

Guidelines for Euthanasia of Rodents Using Carbon Dioxide

Rodents must be euthanized by trained personnel using appropriate technique, equipment and agents. This is necessary to ensure a painless death that satisfies research requirements. Death should be induced as painlessly and quickly as possible. Upon completion of the procedure, death must be confirmed by an appropriate method, such as ascertaining cardiac and respiratory arrest or noting an animal's fixed and dilated pupils. Euthanasia should not be performed in the animal room. The euthanasia method must be appropriate to the species, approved in the animal study proposal and conform to the most recent Report of the AVMA Panel on Euthanasia.

CO_2 inhalation is the most common method of euthanasia used at NIH for mice, rats, guinea pigs and hamsters. A few important aspects of this procedure are:

1. The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber: all animals in the chamber must be able to make normal postural adjustments.

2. Compressed CO_2 gas in cylinders is the only recommended source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide at the rate of 10-20% of the chamber volume per minute so as to optimize reduction in distress. (For a 10-liter volume chamber, use a flow rate of approximately 1-2 liter(s) per minute.) After the animals become unconscious, the flow rate can be increased to minimize the time to death. Sudden exposure of conscious animals to carbon dioxide concentrations of 70% or greater has been shown to be distressful.[1]

3. Animals should be left in the container until clinical death has been ensured.

4. Neonatal animals (up to 14 days of age) are resistant to the effects of CO_2, therefore, alternative methods are recommended.[3] Carbon dioxide may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (i.e. decapitation using sharp blades).

5. If an animal is not dead following CO_2 exposure, another approved method of euthanasia (e.g. decapitation) must be added while the animal is under CO_2 narcosis to assure death. Please refer to Appendixes 1 and 2 of the 2000 Report of the AVMA Panel on Euthanasia[2] for additional recommended methods.

References

1. Danneman PJ, Stein S, Walshaw SO. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. Lab Anim Sci 1997, 47:376-385.
2. AVMA Panel on Euthanasia. 2000 Report of the AVMA Panel on Euthanasia. J Am Vet Med Assoc 2001, 218:669-696.
3. Guidelines for the Euthanasia of Mouse and Rat Fetuses and Neonates. NIH Animal Research Advisory Committee, 2001 (see web site: http://oacu.od.nih.gov/ARAC/euthmous.htm).

Approved - 9/12/01
Revised - 10/9/02

Guidelines for Euthanasia of Mouse and Rat Fetuses and Neonates

GUIDELINES FOR THE EUTHANASIA OF MOUSE AND RAT FETUSES AND NEONATES

The Report of the AVMA Panel on Euthanasia provides limited recommendations for the euthanasia of prenatal or neonatal animals.[1] The following guidelines are suggested to assist individual Animal Care and Use Committees at the NIH in reviewing proposals which involve the use of rodent fetuses or neonates.

Fetuses:

a) Fetuses up to 14 days in gestation: Neural development at this stage is minimal and pain perception is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to loss of blood supply and non-viability of fetuses at this stage of development.

b) Fetuses 15 days in gestation to birth: the literature on the development of pain pathways suggests the possibility of pain perception at this time. Whereas fetuses at this age are resistant to inhalant anesthetics including CO_2, euthanasia may be induced by the skillful injection of chemical anesthetics. Decapitation with surgical scissors, *or* cervical dislocation are acceptable physical methods of euthanasia. Rapid freezing, without prior anesthesia, as a sole means of euthanasia is not considered to be humane. Animals should be anesthetized prior to freezing. When chemical fixation of the whole fetus is required, fetuses should be anesthetized prior to immersion in or perfusion with fixative solutions. Anesthesia may be induced by hypothermia[2] of the fetus, by injection of the fetus with a chemical anesthetic, or by deep anesthesia of the mother with a chemical agent that crosses the placenta, e.g., pentobarbital. The institute veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents. When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother must ensure rapid death of the fetus.

Neonates:

a) Up to 14 days of age: Acceptable methods for the euthanasia of neonatal mice and rats include: injection of chemical anesthetics (e.g., pentobarbital), decapitation, or cervical dislocation. Additionally, these animals are sensitive to inhalant anesthetics; e.g., halothane or isoflurane (used with appropriate safety considerations). Immersion in liquid nitrogen may be used only if preceded by anesthesia. Similarly, anesthesia should precede immersion or perfusion with chemical fixatives. Anesthesia may be induced by inhalant or injectable anesthetics; the institute veterinarian should be consulted for appropriate agents and dosages. Alternatively, when adequately justified, hypothermia[2] may be used to induce anesthesia in pups six days of age or less.

b) Older than 14 days: Follow guidelines for adults.

In all cases, the person performing the euthanasia must be fully trained in the appropriate procedures.

Approved - February 12, 1997
Revised - November 10, 1998
Revised - March 27, 2002

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[1] "When ovarian hysterectomies are performed, euthanasia of feti should be accomplished as soon as possible after removal from the dam. Neonatal animals are relatively resistant to hypoxia." 2000 Report of the AVMA Panel on Euthanasia, JAVMA 218:688.

[2] Phifer CB, Terry LM. 1986. Use of hypothermia for general anesthesia in preweanling rodent. Physiol & Behav 38:887-890.

NINDS/NIDCD ACUC Policy on Euthanasia of Mouse and Rat Fetuses and Neonates by Decapitation

NINDS/NIDCD Animal Care and Use Committee (ACUC) Policy for

Euthanasia of Mouse and Rat Fetuses and Neonates by Decapitation

REFERENCE

ARAC *Guidelines for the Euthanasia of Mouse and Rat Fetuses and Neonates* (Revised 11/10/98)

PURPOSE

The NINDS/NIDCD ACUC recognizes the need to establish a policy on procedures for euthanasia by decapitation with scissors instead of using a guillotine in mouse/rat pups less than 14 days of age.

PROCEDURE

Rats — Newborn to P12

Mice — Newborn to P14

At these young ages, animals are extremely active. Such activity may hamper correct placement of the animal into a guillotine. Surgical scissors (with at least 4cm blades) can be used to efficiently decapitate the animal. Two methods are currently in use. In the first method, the animal is held gently by its snout and body weight causes limbs and trunk to hang away from the neck. This allows the investigator to make a rapid and clean cut, decapitating the animal immediately. In the second method, the animal is held snuggly in one's hand with the neck (close to the forelimbs) held between the forefinger and thumb. Practice holding the animal in this manner will be required. This is necessary to achieve a rapid, clean, scissors cut for immediate decapitation.

NINDS/NIDCD ACUC Policy on Euthanasia Using the Method of Cervical Dislocation

NINDS/NIDCD Animal Care and Use Committee (ACUC) Policy on Euthanasia Using the Method of Cervical Dislocation

REFERENCES:

1. PHS Policy on Humane Care and Use of Laboratory Animals (Section IV.C.1.f & g).

2. The 2000 Report of the AVMA Panel on Euthanasia (p.682).

3. NINDS/NIDCD ACUC Minutes (May 10, 2001).

PURPOSE:

The NINDS/NIDCD ACUC recognizes the need to establish a formal policy on who can perform euthanasia using cervical dislocation without anesthesia when the need to use the method is scientifically justified in an animal study protocol.

PROCEDURE:

1. The investigator/s who will perform the procedure must be identified in Section J of the animal study protocol.

2. The degree of proficiency of the investigator designated to perform cervical dislocation must be ascertained, preferably during the pre-ACUC review of the protocol.

3. The PI and the investigator designated to perform cervical dislocation must meet with NINDS Animal Health and Care Section (AHCS) veterinarian or an experienced member of the ACUC to demonstrate the investigator's skill in the procedure.

4. Investigators who do not have a high degree of proficiency must receive training until they are able to perform the procedure in a manner that is consistently humane.

5. The protocol will receive its final approval after the completion of the training.

Animal Health and Care Section (AHCS) SOP 301: Maintenance of Guillotines

ANIMAL HEALTH AND CARE SECTION
NINDS

SOP 301
August 1998

SUBJECT: MAINTENANCE OF GUILLOTINES

A. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to ensure that guillotines are kept in working order. The blade movement should be smooth with no perceptible binding or resistance. The blade must be rust-free, clean, sharp and decapitate with minimal force.

B. APPLICABILITY

The contents of this SOP apply to all personnel in the NINDS/NIDCD who have approved animal studies authorizing the use of a guillotine.

C. GENERAL INFORMATION

1. The guillotines should be marked with a number or other identification.

2. Guillotines in the 36 SAF should not be moved from room to room. If you need to transfer a guillotine to a different room, the guillotine should be sanitized before moving and before returning to the original room.

3. Log books for guillotines are located in each procedure room and these books should be notated with each use. In laboratories, a log book should be located near the guillotine and each use should be notated.

D. RESPONSIBILITIES

1. Anyone using a guillotine should ensure it is in good condition prior to its use. If a guillotine in the 36 SAF is not working properly, please report this to the Floor Leader (496-3529) or Facility Manager (496-7108) so it can be repaired.

2. Personnel using a guillotine are responsible for proper cleaning after use.

3. The Floor Leader will ensure that 36 SAF guillotines are lubricated as needed with silicon.

4. Investigators may contact the Floor Leader if they want their guillotine lubricated by AHCS staff.

5. The Floor Leader/Facility Manager will ensure the 36 SAF and ACRF guillotines are rotated for sharpening at a minimum of every twelve months or more often if needed. Depending on species and number of animals, investigators may need to have the blades on their laboratory guillotines sharpened every six months.

6. Persons responsible for guillotine(s) must maintain a log book. The log book should include the following information:

    a) Identification number of the guillotine;

    b) Room location;

    c) Person responsible for maintenance and repair;

    d) Useage tracking (i.e. date, species and number of animals euthanized);

    e) Date of blade sanitization and sharpening (to be done annually or more frequently if use requires).

E. PROCEDURE

1. Daily Use

    a) Before using the guillotine, check for rust, blade sharpness, ease of blade movement and cleanliness.

    b) After use, rinse the entire guillotine under fast-running cold water to remove any blood and tissues.

    c) The base should be carefully scrubbed with disinfectant to reduce gross contamination.

    d) A final alcohol rinse will assure evaporation and reduce the need to hand-dry the equipment. The guillotine should be turned upside down with the blades opened to facilitate drying.

2. Maintenance

    a) The Animal Health and Care Section (AHCS) will maintain the guillotines in the 36 SAF.

b) If an investigator needs to have a guillotine sanitized and/or blade sharpened, the AHCS will assist in this service. **All blades must be sharpened annually, as a minimum.**

c) Bring the guillotine to be serviced to AHCS Floor Leader/Facility Manager and provide a CAN number for billing of sharpening service.

d) If an investigator needs a replacement guillotine while their guillotine is being sharpened, contact Floor Leader/Facility Manager to receive a temporary replacement.

e) Only qualified people should take a guillotine apart. The Floor Leader/Facility Manager can provide assistance.

f) Prior to sharpening or more often if needed, the guillotine should be taken apart and sanitized by running the guillotine through the tunnel washer in a basket. Contact the Floor Leader/Facility Manager to request sanitization.

1) Frequency of sharpening depends on both frequency of use and the species euthanized.

2) For example, using the guillotine only 1-2 times per month may require less frequent sharpening than heavy use (many animals, 4-6 times per month)

3) Species will also influence how often blades should be sharpened. For example, 10-20 mice euthanized 2-3 times per month may require less frequent sharpening of blades than 5-10 guinea pigs 2 times per month.

4) In short, use common sense. The best cutting blade dulls after use.

g) After sanitizing, the guillotine is taken to BEIP, Bldg.13, 3rd Floor ATTENTION: Mr. Midjette, 496-5197/ 5195 for sharpening.

h) The sharpening should take no longer than two weeks. Contact Mr. Midjette if you have not been notified to pick up the guillotine after two weeks.

4. Replacement

a) Mr. Midjette and his staff will determine if the blades can be sharpened or should be replaced.

b) If the blades cannot be sharpened, the guillotine should be taken apart and the blades disposed of in a sharps container; place the stand in the dumpster, NOT MPW.

5. The NINDS/NIDCD Animal Care and Use Committee (ACUC) will ask to review your guillotine log book during the semi-annual review of labs, animal holding facilities, and program review.

F. POINTS OF CONTACT

Facility Manager	496-7108
Floor Leader	496-3529
Administrative Tech	496-9354

NINDS/NIDCD Animal Care and Use Committee (ACUC) Policy Regarding MAP, RAP, HAP, or PCR Testing of Tissue Cultures and Biologics

NINDS/NIDCD ACUC Policy Regarding MAP, RAP, HAP or PCR Testing of Tissue Cultures and Biologics

REFERENCE

Kilham L and LJ Olivier. A Latent Virus of Rats Isolated in Tissue Culture. Virology 1959; 7:428-437.

Dykewicz DA, Dato VM, Fisher-Hoch SP, Howarth MV, et al. Lymphocytic Choriomeningitis Outbreak Associated with Nude Mice in a Research Institute. JAVMA 1992; 267(10):1349-1353.

Lipman NS, Perkins S, Nguyen H, et al. Mousepox Resulting from Use of Ectromelia Virus-Contaminated, Imported Mouse Serum. Comp Med 2000; 50(4):426-435.

Compton S.R. and Riley L.K. Detection of Infectious Agents in Laboratory Rodents: Traditional and Molecular Techniques. Comp. Med., 2001;51(2):113-119.

PURPOSE

Details of passage history of respective tissue or tumor cell lines, such as microbiological conditions of the animals used, are frequently unknown; similar problems exist for commercial biologic materials. Testing of tissue cell lines and/or murine-derived biological products destined for use in animals is important for protection of both the animal colony and employees. Either of the following methods satisfies the requirement for virus testing of tissue cultures and biologics.

PROCEDURE

Method 1. The Mouse Antibody Production (MAP) test is one of the most sensitive methods for detection of adventitious murine viruses, as is the Rat Antibody Production (RAP) and Hamster Antibody Production (HAP) tests for rat and hamster viruses, respectively. These are traditional tests considered essential for the safety assessment of murine cell derived and ascites-based products.

- Minimum Testing Requirements
    - **Mouse Antibody Production (MAP)**, a non-GLP, 12 virus panel: Sendai, MHV, PVM, Reo3, GDVII, Ectromelia, MVM, Polyoma, LDHV, MAd, EDIM, and LCM.
    - **Rat Antibody Production (RAP)**, a non-GLP, 8 virus panel: Toolans H-1, GDVII, KRV, PVM, Reo3, Sendai, RCV/SDA, and Hantaan.
    - **Hamster Antibody Production (HAP)**, a non-GLP, 5 virus panel: Reo3, PVM, Sendai, SV5, and LCM.
- Cell lines or tissue cultures must be tested prior to introducing the material into an animal facility **or** if the cell-line has been passed through animals since testing.
- When testing hamster lines, it is also strongly recommended that the MAP assay be performed in addition to the HAP assay since hamsters may be susceptible to some of the agents of the MAP assay.
- Cell lines or tissues must be tested again whenever passaged into an animal and then recollected.

Method 2. PCR has begun to replace the MAP test as the preferred test for detecting viral contaminants in biological materials. A panel of up to 18 Polymerase Chain Reactions (PCR)

and Reverse Transcriptase PCRs (RT-PCR) can be performed on nucleic acids extracted from biological material. A direct comparison of the sensitivity of MAP and PCR-based testing for the detection of 11 murine viruses indicated that PCR-based testing was more sensitive than MAP testing for 8 of the viruses, while detection of the other 3 viruses was comparable in both tests. MAP testing is renowned for false positive results due to nonspecific reactions of mouse serum in serological assays. PCR-based testing avoids this problem. However, the sensitivity of PCR, one of its greatest advantages, is also one of its greatest disadvantages. Contamination of negative samples with only minute amounts of nucleic acids from a positive sample can result in a false positive results.

- **Advantages of PCR**
  - Rapid response time: 3-5 days compared to 6-8 weeks for MAP/RAP/HAP
  - Increased sensitivity
  - Cost
  - Alternative to use of animals
  - Less potential risk of exposure of personnel to zoonotic agents, such as LCMV or hantavirus. The infectivity of agents present in biological material is rapidly destroyed during nucleic acid extraction procedures, minimizing risk of exposure.

For information on how to submit biological materials for testing, contact the AHCS.